STUDIES ON THE PHARMACOLOGY OF CAROTID BODY CHEMORECEPTORS

Daniel S. McQueen

D.Sc.
University of Edinburgh
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CONTENTS

STATEMENT UNDER REGULATION 1.1.4.

(a) Part of the work in Papers 1-5 was presented in my PhD thesis (University of London, 1970)

(b) I was responsible for initiating and performing the major part of the work for most of the papers presented. In the co-authored publications valuable contributions were made by PhD students, research technicians, or other colleagues. Papers in which my contribution accounted for less than half the total work are: 6, 31, 46, 48, 50.

A general policy of listing authors' names in alphabetical order has been adopted, and was a Journal requirement for most of the publications.

Signed (D.S. McQueen)

ABSTRACT

The work presented in this thesis arose from an interest in the pharmacology of peripheral arterial chemosensors and in the mechanism of chemoreception. Publications have been grouped into five sections, with each section representing different aspects of the work.

Section 1 is concerned with the study of various reflexes evoked by stimulating the carotid chemoreceptors with drugs and also includes the finding that capsaicin reduces carotid chemosensitivity.

Section 2 contains papers which describe the results of neuropharmacological experiments performed to characterise the acetylcholine (ACh) receptors of the carotid body. Quantitative analysis of the data provided objective evidence that the increase in chemosensory discharge evoked by ACh results from actions on a nicotinic ACh receptor similar to that present in autonomic ganglia. The discovery was made that ACh causes chemodepression in rabbits via actions on muscarinic ACh receptors. Dopamine (DA) depresses chemoreceptor discharge in cats and rabbits, and the use of selective agonists and antagonists revealed that this effect is mediated via D_2 DA receptors.

Section 3 relates to the discoveries that the polypeptide substance P can affect chemosensory discharge and is present in the cat carotid body, and that opioid peptides are potent inhibitors of chemoreceptor activity. The greater part of the chemodepression evoked by [Met]-enkephalin can be attributed to actions on delta opioid receptors. The interaction between various peptides and amines has been examined, and the 5-hydroxytryptamine receptors of the carotid body have been characterised using selective antagonists.

Section 4. Prostaglandins were found to be capable of stimulating respiration, but appear not to have any direct action on the peripheral chemoreceptors. Adenosine and ATP do affect chemoreceptor discharge, and the adenosine receptor involved has been partially characterised by the use of selective agonists and antagonists.

Section 5 includes miscellaneous publications on work which was indirectly related to the study of chemoreceptors. The last paper is a review of chemoreceptor pharmacology.

PUBLICATIONS

SECTION 1 - CHEMORECEPTOR REFLEXES

1 McQueen, D.S. & Ungar, A. (1971) On the direct and crossed components of reflex responses to unilateral stimulation of the carotid body chemoreceptors in the dog. J. Physiol. 219, 1-16.

2 McQueen, D.S. & Ungar, A. (1969) The direct and crossed vagal components of the reflex bradycardia following stimulation of the carotid body chemoreceptors in the dog. J. Physiol. 202, 30-31P.

3 McQueen, D.S. & Ungar, A. (1969) The direct and crossed components of the reflex vasoconstriction in the hind limbs of the dog following stimulation of the carotid body chemoreceptors. J. Physiol. 203, 48-49P.

4 McQueen, D.S. & Ungar, A. (1969) The direct and crossed components of the reflex increase in phrenic nerve activity following stimulation of the carotid body chemoreceptors in the dog. J. Physiol. 204, 131-132P.

5 McQueen, D.S. & Ungar, A. (1970) The direct and crossed components of the reflex release of catecholamines from the adrenal medulla of the dog following stimulation of the carotid body chemoreceptors. J. Physiol. 207, 20-21P.

6 McQueen, D.S. & Ungar, A. (1971) Can drugs replace hypoxic drive in respiratory depression? Br. J. Pharmac. 43, 449-450P.

7 Bond, S.M., Cervero, F. & McQueen, D.S. (1982) Treatment of newborn rats with capsaicin reduces baro- and chemo-reflex activity. J. Physiol. 325, 15P.

8 Bond, S.M., Cervero, F. & McQueen, D.S. (1982) Influence of neonatally administered capsaicin on baroreceptor and chemoreceptor reflexes in the adult rat. Br. J. Pharmac. 77, 517-524.

9 McQueen, D.S. & Eyzaguirre, C. (1974) Effects of temperature on carotid chemoreceptor and baroreceptor activity. J. Neurophysiol. 37, 1287-1296.

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14 Docherty, R.J. & McQueen, D.S. (1978) Inhibitory action of dopamine on cat carotid chemoreceptors. J. Physiol. 279, 425-436.

15 McQueen, D.S. (1978) Effects of methacholine on the carotid chemoreceptors. Q. J. Exp. Physiol. 63, 171-178.

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17 McQueen, D.S. (1980) Effects of acetylcholine and sodium cyanide on cat carotid baroreceptors. Br. J. Pharmac. 69, 433-440.

18 McQueen, D.S. (1980) Effects of dihydroβerythroidine on the cat carotid chemoreceptors. Q. J. Exp. Physiol. 65, 229-237.

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20 McQueen, D.S. (1984) Effects of selective dopamine receptor agonists and antagonists on carotid body chemoreceptor activity. In: 'Proceedings of the VII International Meeting on Arterial Chemoreceptors' pp. 325-333, Ed. Pallot, D.J. Croom Helm.

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22 McQueen, D.S., Mir, A.K., Brash, H.M. & Nahorski, S.R. (1984) Increased sensitivity of rabbit carotid body chemoreceptors to dopamine after chronic treatment with domperidone. Eur. J. Pharmacol. 104, 39-46.

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SECTION 3 - POLYPEPTIDES and 5-HYDROXYTRYPTAMINE

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27 McQueen, D.S. & Ribeiro, J.A. (1980) Inhibitory actions of methionine-enkephalin and morphine on the cat carotid chemoreceptors. Br. J. Pharmac. 71, 297-305.

28 McQueen, D.S. (1981) Effects of some polypeptides on carotid chemoreceptor activity. In: 'Arterial Chemoreceptors, Proceedings of the IV International Meeting' pp. 299-308, Ed. Belmonte, C. et al. Leicester University Press.

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35 Kirby, G.C. & McQueen, D.S. (1984) Effects of substance P on responses of cat carotid body chemoreceptors to dopamine, noradrenaline and 5-hydroxytryptamine. Br. J. Pharmac. 83, 455P.

SECTION 4 - PROSTAGLANDINS, ADENOSINE and ATP

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39 McQueen, D.S. (1974) The effects of some prostaglandins on respiration in anaesthetized cats. Br. J. Pharmac. 50, 559-568.

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SECTION 5 - REVIEWS and MISCELLANEOUS TOPICS

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SECTION 1

CHEMORECEPTOR REFLEXES

PAPERS 1 - 8

J. Physiol. (1971), **219**, *pp.* 1–16
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ON THE DIRECT AND CROSSED COMPONENTS OF REFLEX RESPONSES TO UNILATERAL STIMULATION OF THE CAROTID BODY CHEMORECEPTORS IN THE DOG

By D. S. McQUEEN and A. UNGAR

From the Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

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SUMMARY

1. In dogs under chloralose-urethane anaesthesia the chemoreceptors of the two carotid bodies were separately stimulated.

2. The distribution of three primary reflex responses to carotid body stimulation was studied: parasympathetic bradycardia, sympathetic vasoconstriction, and increase in somatic phrenic nerve activity.

3. The reflex bradycardia evoked by either carotid body was mediated by both vagus nerves, but when either vagus was blocked a greater response could be obtained from the contralateral than from the ipsilateral carotid body.

4. The reflex vasoconstriction evoked by either carotid body was seen in both hind limbs, with no predominance in either limb.

5. The reflex increase in phrenic nerve activity evoked by either carotid body was seen in both phrenic nerves, with no predominance in either nerve.

INTRODUCTION

Among the primary reflex responses to carotid body chemoreceptor stimulation in the dog are hyperpnoea, bradycardia due mainly to increased vagal tone, and systemic vasoconstriction due entirely to increased sympathetic tone (Daly & Scott, 1958; Bernthal, Motley, Schwind & Weeks, 1945). In spontaneously breathing dogs the primary bradycardia and vasoconstriction may be masked by secondary effects of the increase in pulmonary ventilation (Daly & Scott, 1962, 1963). A similar situation is found in the cat (Scott, 1966) and in the rabbit (Korner & Edwards, 1960).

Fedorchuk (1957) found that, after ablation of one carotid body in decerebrate cats, intravenous injections of chemoreceptor stimulant

drugs caused an increase in impulse traffic in the phrenic nerve on the side of the intact carotid body and no increase on the side of the ablated carotid body. Fedorchuk (1954) also found that stimulation of one carotid body caused a reflex release of catecholamines predominantly or solely from the adrenal gland on the same side. Nazarenko (1958) studying the reflex bradycardia on carotid body stimulation in decerebrate cats concluded that the right carotid body exerts its effect entirely through the right vagus nerve, while the left carotid body acts through both vagi.

We have studied the distribution between the two sides of the body of the somatic respiratory, the parasympathetic cardiac and the sympathetic vascular effects of the stimulation of one carotid body at a time in dogs. Our results indicate that the parasympathetic effect is mainly ipsilateral, while the sympathetic and somatic effects are evenly distributed on the two sides.

Preliminary accounts of this work have been published (McQueen & Ungar, 1969*a*, *b*, *c*).

METHODS

Mongrel dogs of either sex weighing between 6·8 and 21·5 kg, and two cats weighing 3·0 and 3·5 kg respectively were used.

Anaesthesia. Dogs were premedicated with morphine sulphate (1 mg/kg subcutaneously) and anaesthetized 30 min later with a mixture of α-chloralose (55 mg/kg) and urethane (550 mg/kg) injected intravenously as a solution containing 2·5 % α-chloralose and 25 % urethane in a mixture of equal volumes of 0·9 % aqueous sodium chloride and polyethylene glycol 200. To maintain anaesthesia a 2 % solution of α-chloralose was infused at a rate adjusted according to the state of the animal's reflexes.

One cat was anaesthetized by an intraperitoneal injection of pentobarbitone (25 mg/kg) and the other was decerebrated at the intercollicular level under ether anaesthesia.

Respiration. In all animals the trachea was cannulated in the neck.

The lungs of eight dogs were artificially ventilated with a 60 % oxygen 40 % nitrogen mixture by a Starling 'Ideal' pump. Their chests were opened by splitting the sternum in the mid line, and held open by a self-retaining retractor after ligature of the internal thoracic vessels.

In twenty dogs under artificial ventilation decamethonium iodide (0·25 mg/kg) was given intravenously to prevent respiratory movements when the chemoreceptors were stimulated.

Blood from a cannulated femoral artery was sampled at 15 min intervals throughout the experiment. The $P_{a.CO_2}$, $P_{a.O_2}$ and pH were estimated by means of a Radiometer PA 927 gas monitor. Arterial P_{CO_2} was held between 30 and 35 torr by adjustment of the stroke of the respiratory pump which ran at 20 rev/min. The plasma bicarbonate concentration was held between 20 and 25 mM by intravenous injections of molar sodium bicarbonate solution, the base deficit being calculated from the nomogram of Singer & Hastings (1948).

The $P_{a.O_2}$ was always above 150 torr. Under these conditions we assumed that there was no resting chemoreceptor tone, so that the results should not have been com-

plicated by withdrawal of resting tone when the vagi or carotid sinus nerves were cut or blocked.

Elimination of secondary reflexes from the lungs. In the animals with open chests the thoracic vagal trunks were cut, on the left side between the aorta and the left pulmonary artery, and on the right side at the level of the azygos vein. This procedure eliminates the pulmonary branches of the vagi but does not affect the cardiac branches (Daly & Scott, 1963).

In animals with intact chests, where cardiac reflexes were not being studied, both vagosympathetic trunks were cut in the neck.

Elimination of reflexes from the bladder. Each animal's bladder was catheterized to prevent progressive filling with the resulting influence on autonomic reflexes (Oberholzer, 1963).

Blood pressure compensation. In five dogs with open chests a blood pressure compensator was connected to the left subclavian artery in order to reduce fluctuations in arterial pressure on chemoreceptor stimulation. The compensator consisted of an inverted conical flask of 1 litre capacity containing about 200 ml. 0·9 % sodium chloride solution. The outlet was connected to the artery by wide bore silicone rubber tubing, and the air space in the flask connected to a source of compressed air set to the animal's resting arterial pressure.

Temperature control. A thermistor probe (Standard Telephones & Cables type F23) was inserted into the animal's rectum, and connected through an A.E.I. 'Sunvette' temperature controller to an infra-red heater. The animal's temperature was held at $37 \pm 0{\cdot}5°$ C.

Stimulation of the carotid body chemoreceptors. Nylon catheters (0·75 mm o.d.) were inserted through both superior thyroid arteries, their tips lying in the common carotid arteries about 3 cm upstream of the origins of the superior thyroid arteries. Each carotid body was independently stimulated by infusion through the appropriate catheter, with a Watson Marlow MHRE pump, of a solution of suberyl dicholine di-iodide (83 μM) in a phosphate buffer at pH 7·4. The infusions lasted 10 sec, and were separated by intervals of 5 min, the two carotid bodies being stimulated alternately. The amount of suberyl dicholine di-iodide (SDC) infused during a stimulus varied from 3 to 58 n-mole (2–35 μg), the usual effective dose being 30 n-mole (18 μg).

SDC has peripheral nicotinic actions but no central nervous effects at the dosage used. It is rapidly metabolized by pseudocholinesterase (Anichkov & Belen'kii, 1963). We have found that this time cycle of dosage can be followed for several hours without tachyphylaxis in the reflex responses. This confirms similar findings in the cat (Mikhel'son, Rybovlev, Gorelik & Dardymov, 1957).

Block of conduction in the vagi. Each vagosympathetic trunk in the neck was dissected and placed on a cooling device (Daly, Hazzledine & Ungar, 1967). The two nerves were cooled in turn to between 0 and $-2°$ C during cycles of alternate carotid body stimulation.

The bradycardia obtained by electrical stimulation of the cut vagus nerve was abolished on cooling to 5° C, and returned to its previous magnitude on rewarming even after cooling to $-4°$ C.

Recording procedure. A Honeywell 2106 twelve channel ultra-violet oscillograph was used. Honeywell M400/350 galvanometers gave a frequency response flat to 320 Hz. Kodak Linagraph 1843 paper was used.

Arterial pressure. Arterial pressure was measured by means of a Bell and Howell L222 transducer from a catheter (2 mm o.d.) in either a femoral or a radial artery. The output from the transducer was amplified by a Fenlow AD2000 amplifier and recorded on a channel of the oscillograph.

Measurement of changes in limb vascular resistance. A nylon catheter (2 mm o.d.) was inserted through each femoral artery just below the inguinal ligament so that its tip lay in the abdominal aorta. The catheter was connected by a loop of silicone rubber tubing, which passed through a Watson Marlow MHRE pump, to a cannula inserted caudally into the same femoral artery. A tape was tied tightly around the limb between the catheter and the cannula, avoiding the femoral vein. This perfusion system provided a constant flow against arterial pressures up to 250 mm Hg.

The time taken for blood to traverse the perfusion tube was about 12 sec at the pump speed usually used.

The perfusion pressure in each limb was recorded from a 'Y' piece in the perfusion tubing in a similar way to that described for systemic arterial pressure. As the limbs were perfused at constant flow, pressure changes were taken to be proportional to changes in vascular resistance.

Cardiac period (R–R interval). The electrocardiogram was recorded from chest leads chosen to give prominent R waves. A trigger circuit was set so that each R wave reset a linear ramp generator (Computing Techniques SA 6). The height of each ramp, recorded on a channel of the oscillograph, was proportional to the R–R interval. The mean cardiac period during the first 10 sec of each test of carotid body stimulation was compared with the mean cardiac period before, and after stimulation.

Electrical activity in the phrenic nerves. Each phrenic nerve was cut between ligatures at the level of the seventh cervical transverse process, and placed on bipolar platinum electrodes in a pool of mineral oil. Impulses were amplified by a differential input FET amplifier passing a band of frequencies between 10 and 1 KHz.

Activity above a threshold set to exclude the resting noise was averaged with a time constant of 20 msec, and recorded on a channel of the oscillograph.

The amplitude of the averaged record was taken as a measure of the impulse traffic in an inspiratory burst. Changes during carotid body stimulation were calculated as the mean increase in amplitude of inspiratory peaks expressed as a percentage of the mean resting amplitude before and after stimulation.

Drugs. The following drugs were used: α-chloralose (B.D.H. or Koch Light Laboratories Ltd), polyethylene glycol 200 (B.D.H.), ethyl carbamate (urethane) (B.D.H. or May and Baker Ltd.), decamethonium iodide (K and K Labs Inc., New York), morphine sulphate (Macarthys Ltd), atropine sulphate (B.D.H.), suberyl dicholine di-iodide (kindly prepared by Dr J. N. T. Gilbert, Department of Pharmaceutical Chemistry, School of Pharmacy, London University) and guanethidine sulphate (Ciba).

RESULTS

Reflex bradycardia. We carried out preliminary experiments in order to establish

(i) whether the changes in heart rate recorded were due entirely to stimulation of receptors on the side into which the stimulant was infused.

(ii) whether the changes in heart rate were mediated entirely by parasympathetic fibres in the two vagus nerves.

In two dogs tests of carotid body stimulation were carried out on both sides before and after section of one carotid sinus nerve. In both animals the cardiac response to stimulation on the denervated side was abolished, while the response to stimulation on the intact side was unaffected.

In two dogs tests of carotid body stimulation were carried out before and after intravenous administration of atropine sulphate (180 n-mole/kg or 125 μg/kg and 360 n-mole/kg or 250 μg/kg). The reflex bradycardia was abolished, while the concomitant vasoconstriction in the hind limbs was unaffected.

In five dogs tests of carotid body stimulation were carried out before and after division of both vagosympathetic trunks in the neck. Reflex bradycardia was abolished in all of these.

Twenty-eight tests of carotid body stimulation were carried out on six dogs with intact chests artificially ventilated and paralysed with decamethonium iodide. In four out of thirteen tests the reflex slowing of the heart was potentiated by cooling the left vagus, and in six out of fifteen tests it was potentiated by cooling the right vagus. We attributed this effect, which invalidates any quantitative comparison between the two sides, to blocked sensory fibres in the vagi from pulmonary stretch receptors, and possibly also from aortic baroreceptors (see Discussion).

Forty-three tests of carotid body stimulation were carried out on seven dogs with open chests after cutting both vagi between the origins of their cardiac and pulmonary branches (see Methods). A typical record of a set of responses is shown in Fig. 1. The results are shown in Table 1. While both vagi were intact, stimulation of either carotid body gave rise to a similar degree of slowing of the heart. While the left vagus was blocked, stimulation of the right carotid body gave rise to greater slowing than did stimulation of the left carotid body in six out of seven dogs. Conversely, while the right vagus was blocked, stimulation of the left carotid body gave rise to greater slowing than did stimulation of the right carotid body in all of the seven dogs. These differences are statistically significant ($P < 0{\cdot}01$). Whichever vagus was blocked, the degree of slowing produced by stimulation of the ipsilateral carotid body was about half that produced by stimulation of the contralateral carotid body. While the left vagus was blocked both the ipsilateral and contralateral responses were about twice the size of the corresponding ipsilateral and contralateral responses obtained while the right vagus was blocked.

Reflex vasoconstriction. As with the reflex bradycardia we carried out preliminary experiments in order to establish

(i) whether the changes in hind limb resistance recorded were due entirely to stimulation of receptors on the side into which the SDC was infused.

(ii) whether the changes were mediated entirely by sympathetic adrenergic fibres.

In three dogs stimulation of each carotid body gave rise to reflex vasoconstriction. After section of one carotid sinus nerve in each dog

vasoconstriction was only seen when the innervated carotid body was stimulated.

In three dogs guanethidine sulphate (3·5 μmole/kg) was injected close-arterially into one hind limb while both hind limbs were perfused at constant flow. A test of chemoreceptor stimulation was carried out before the guanethidine had time to circulate. In each experiment the reflex vasoconstriction previously observed in that limb was abolished, with no effect on the vasoconstriction in the other hind limb.

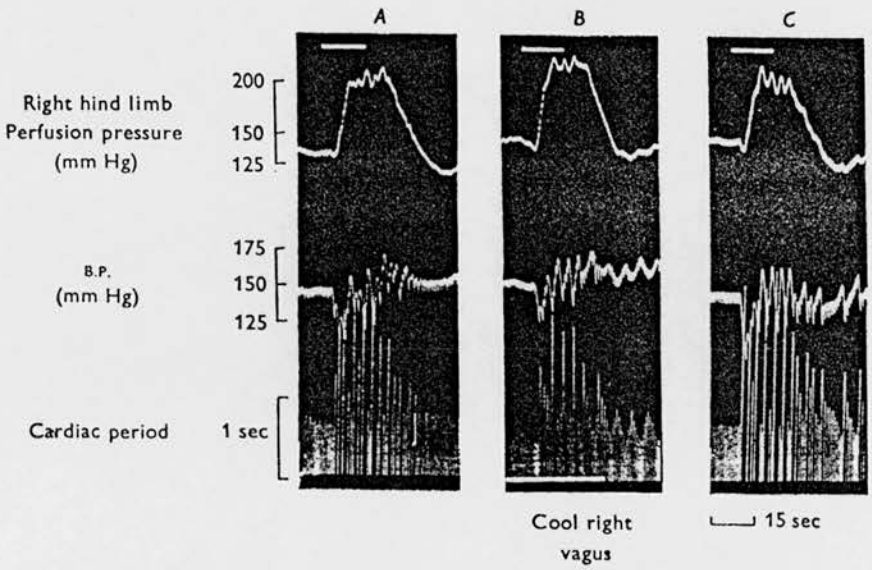

Fig. 1. Reflex responses to stimulation of the right carotid body of a dog. The records from above downwards show arterial pressure in the right hind limb, perfused at constant flow, mean systemic arterial pressure, and cardiac period (R–R interval). The marker at the top indicates the period of stimulation by infusion of SDC into the right common carotid artery. The responses before, during and after cold block of the right vagosympathetic trunk in the neck are shown in blocks *A*, *B* and *C* respectively.

Sixty-six tests of carotid body stimulation were carried out in eight dogs, artificially ventilated and paralysed with decamethonium iodide, after cutting both vagosympathetic nerves in the neck. The results are shown in Table 2. Whichever carotid body was stimulated, the difference between the means of the responses in the two hind limbs is smaller than their standard errors.

In two dogs both forelimbs were similarly perfused at constant flow. Whichever carotid body was stimulated, a balanced vasoconstriction was seen in both forelimbs.

TABLE 1. The effect of stimulation of each carotid body on the cardiac period (R–R interval). Experiments on dogs with open chests, artificially ventilated, the cervical vagi being cooled in turn

A

	Both vagi intact. Cardiac period (msec)			Left vagus cooled. Cardiac period (msec)			Right vagus cooled. Cardiac period (msec)		
		CB stimulated			CB stimulated			CB stimulated	
Experiment	Control	Left	Right	Control	Left	Right	Control	Left	Right
1	400	830	750	470	750	1280	350	470	450
2	360	2000	1840	320	1020	1460	340	1220	720
3	560	710	830	450	490	520	360	380	360
4	500	1460	1920	480	1080	1680	540	1410	960
5	730	1180	1280	540	630	1090	740	1060	810
6	380	710	730	340	620	800	350	430	400
7	530	910	840	460	610	610	430	500	460
Means ± S.E.	490 ± 50	1110 ± 180	1170 ± 190	440 ± 30	740 ± 80	1060 ± 170	440 ± 60	780 ± 160	590 ± 80

B

	Animal		Both vagi intact. Increase in cardiac period (msec). CB stimulated		Left vagus cooled. Increase in cardiac period (msec). CB stimulated		Right vagus cooled. Increase in cardiac period (msec). CB stimulated	
Experiment	Sex	Weight (kg)	Left	Right	Left	Right	Left	Right
1	F	11	430	360	280	810	120	110
2	M	8	1640	1500	700	1140	870	370
3	M	12·8	150	270	40	70	20	0
4	F	14	960	1440	600	1200	870	420
5	M	14·4	280	550	90	550	320	70
6	F	8·2	330	360	280	460	80	50
7	M	14	380	310	150	150	70	30
		Means ± S.E.	590 ± 200	680 ± 210	310 ± 100	630 ± 170	340 ± 140	150 ± 80

TABLE 2. The effect of stimulation of each carotid body on the vascular resistance of both hind limbs perfused at constant flow. Sixty-six tests in eight dogs, artificially ventilated and paralysed with decamethonium iodide, after cutting both vagosympathetic trunks in the neck

Animals		Control			Mean % increase in resistance			
					Left carotid body stimulated		Right carotid body stimulated	
Sex	Weight (kg)	Mean B.P. (mm Hg)	Left leg mean perfusion pressure (mm Hg)	Right leg mean perfusion pressure (mm Hg)	Left left	Right leg	Left leg	Right leg
F	9·5	123	108	122	18·5	22·6	22·5	23·2
F	8·5	140	146	153	9·7	9·3	12·8	9·9
M	9·6	140	160	136	9·8	6·4	6·5	9·5
M	11·8	140	148	142	9·3	12·5	14·0	10·7
M	12·2	115	132	120	75·7	40·9	41·4	51·2
F	11·0	140	129	158	55·9	39·5	22·4	40·9
M	10·2	142	144	151	15·6	18·3	19·3	14·1
M	14·4	113	117	124	19·9	22·7	42·8	30·1
				Means ± S.E.	23·8 ± 3·5	22·1 ± 2·6	25·4 ± 3·7	24·7 ± 3·0

Phrenic neurogram. As in the previous sections, we carried out a preliminary experiment to establish whether the changes in phrenic activity recorded were due entirely to stimulation of receptors on the side into which SDC was infused.

In one dog tests of carotid body stimulation were carried out on both sides before and after section of one carotid sinus nerve while electrical activity was recorded from both phrenic nerves. The reflex increase in activity in both prenic nerves previously evoked from the denervated side was abolished, but the response from the other side was unaffected.

TABLE 3. The effect of stimulation of each carotid body on the averaged electrical activities of both phrenic nerves. One hundred tests in six dogs, artificially ventilated and paralysed with decamethonium iodide, after cutting both vagosympathetic trunks in the neck

Animals		Control		Mean % increase in phrenic activity			
				Left carotid body stimulated		Right carotid body stimulated	
Sex	Weight (kg)	Mean B.P. (mm Hg)	Mean $P_{a.CO_2}$ (torr)	Left phrenic nerve	Right phrenic nerve	Left phrenic nerve	Right phrenic nerve
F	17·5	138	35	86	51	115	70
M	11·8	143	39	62	56	36	37
M	13·4	169	48	53	53	51	50
M	21·5	126	40	39	35	21	21
F	12·6	158	38	24	21	30	26
M	16·0	106	49	36	40	45	63
		Means ± S.E.		48 ± 7	44 ± 4	49 ± 6	45 ± 5

One hundred tests of carotid body stimulation were carried out in six dogs, artificially ventilated and paralysed with decamethonium iodide, after cutting both vagosympathetic nerves in the neck. The results are shown in Table 3. Whichever carotid body was stimulated, the difference between the means of the responses in the phrenic nerves is smaller than their standard errors.

In view of the disparity between our results and those of Fedorchuk (1957), who worked on cats, we repeated our experiments on one anaesthetized and one decerebrate cat. In both of these experiments we obtained uniform increases in activity in the two phrenic nerves, whichever carotid body was stimulated. A record of a pair of tests in the decerebrate cat is shown in Fig. 2.

Systemic arterial pressure. The responses to carotid body stimulation that we studied were accompanied by variable changes in systemic arterial pressure, and these can give rise to secondary reflex changes in heart rate,

vascular tone and respiratory drive by affecting the baroreceptors of the carotid sinuses and aortic arch (Heymans & Neil, 1958).

In the experiments on reflex bradycardia the changes in systemic pressure were biphasic (see Fig. 1) and our measurements of reflex slowing were made before the onset of the sustained rise in pressure. In the other two series of experiments there were simple rises in pressure, as the vagi were

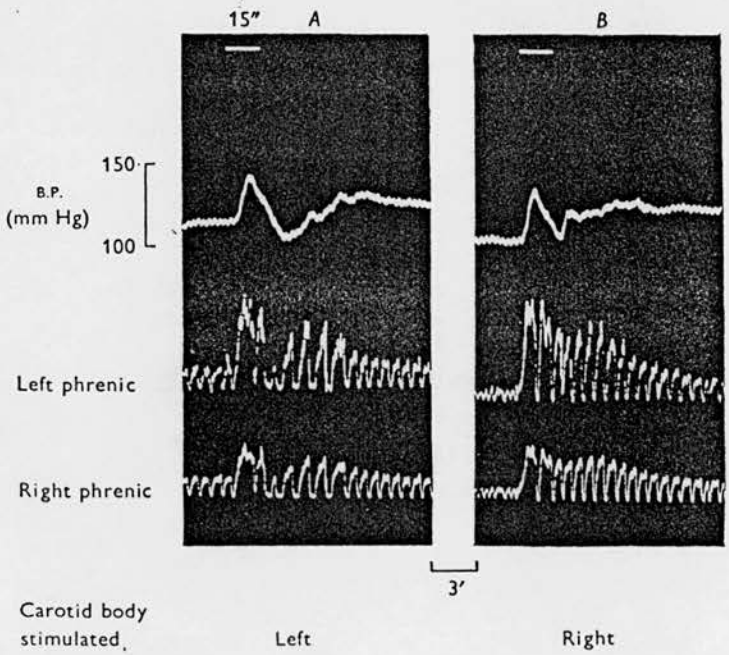

Fig. 2. Reflex responses to stimulation of the left carotid body (*A*) and the right carotid body (*B*) of a decerebrate cat. The records from above downwards show mean systemic arterial pressure, averaged electrical activity in the left phrenic nerve, and averaged electrical activity in the right phrenic nerve.

cut (see Fig. 2). Here our measurements, made in the first ten seconds of the response, should not have been affected by reflex changes secondary to the pressure rise.

In order to establish whether such changes were altering the size of our primary responses, we carried out tests of carotid body stimulation with and without a blood pressure compensator.

In three animals tests of reflex bradycardia, in three animals tests of reflex vasoconstriction, and in one animal tests of reflex increase in phrenic activity were carried out with and without the blood pressure compensator. In none of these experiments did we see any change in the size of the primary reflex responses.

DISCUSSION

The methods used in these experiments have enabled us to study three primary reflex responses to chemoreceptor stimulation, and to stimulate the chemoreceptors of the two carotid bodies separately.

Acetylcholine-like drugs, if used at much higher concentrations than those affecting chemoreceptors, have been shown to stimulate baroreceptor endings in the cat (Diamond, 1955). We exclude the possibility that SDC stimulated the baroreceptors in our experiments for the following reasons:

(i) Jarisch, Landgren, Neil & Zotterman (1952) gave intracarotid injections to cats and dogs of drugs that stimulate both chemoreceptors and baroreceptors. They found that stimulation of chemoreceptor fibres occurred immediately, whereas impulse traffic in baroreceptor fibres increased after delays of at least 15 sec. There would thus be no stimulation of baroreceptors in our experiments during the time when reflex responses were recorded.

(ii) McQueen (1970) studied the problem under similar conditions to those of the present experiments. One carotid body was embolized with lycopodium, using the technique of Heymans & Bouckaert (1933). This procedure did not affect either the cardiac or the vascular reflex responses to occlusion of the common carotid artery, showing that the baroreceptor reflex was intact, nor did it affect the reflex responses to infusion of SDC into the contralateral common carotid artery. The responses to infusion of SDC into the ipsilateral common carotid artery were totally abolished, even at twenty times the greatest dosage used in these experiments.

(iii) Supporting evidence was provided by the observation that intracarotid injections of SDC and sodium cyanide that evoked equal respiratory responses also evoked equal increases in hind limb resistance and cardiac period. Any stimulation of the baroreceptors would have been expected to potentiate the bradycardia, but to counteract the vasoconstriction.

Reflex bradycardia. Pagano (1900) working before the discovery of the sensory function of the carotid bodies, described the bradycardia following the injection of nicotine or of prussic acid into the common carotid artery of a dog. He found that the bradycardia could be much reduced or even abolished by cutting the vagus nerve on the side on which the injection was given. Nazarenko (1958) injected potassium cyanide intravenously into decerebrate cats having one carotid body excised and one vagus nerve cut in the neck, and made a non-parametric comparison of the incidence of bradycardia in the four combinations of unilateral carotid body excision and unilateral vagotomy. He concluded from his results that the reflex

evoked by the right carotid body is mediated only by the right vagus, while the reflex from the left carotid body is mediated by both vagi.

Our results indicate that the reflex from either carotid body is mediated by both vagi, as shown in Fig. 3. Each vagus nerve, however, predominantly mediates the reflex from the carotid body on the same side. We thus seem to agree with Pagano, but not with Nazarenko. This conflict could be due to a species difference between dogs and cats, but it could also be due to two differences in technique:

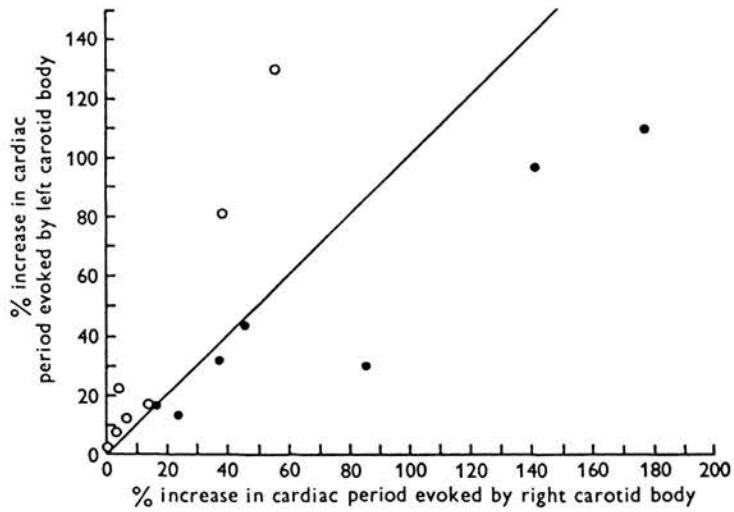

Fig. 3. Spot diagram of ipsilateral against contralateral responses: reflex bradycardia. Graphical representation of the results in Table 1. The percentage increase in cardiac period evoked by stimulation of the left carotid body is plotted against that evoked by stimulation of the right carotid body in each experimental situation. Open circles represent tests in which the right vagosympathetic trunk was cooled; filled circles represent tests in which the left vagosympathetic trunk was cooled. The line of equality is indicated.

(i) Intravenous injections of potassium cyanide would stimulate the aortic bodies as well as the carotid bodies, but sensory inpulses would only reach the brain on the side of the intact vagus.

(ii) The pulmonary inflation reflex, which modifies the response to chemoreceptor stimulation (Daly & Scott, 1962, 1963) would have been active though only on the side of the intact vagus in Nazarenko's experiments, whereas in our experiments it was totally eliminated.

A factor common to our experiments and Nazarenko's is the withdrawal of sensory influence from the heart and great vessels when one or the other vagus nerve is blocked or cut. There are thus different backgrounds of reflex activity in the three situations with one or the other or both

vagi conducting. It is impossible to make quantitative comparisons among the reflexes elicited against these different backgrounds. We have therefore only compared the effects of stimulating the two carotid bodies, in each state of the vagi.

Another possible factor is a component of withdrawal of sympathetic tone in the reflex bradycardia. We studied the reflex bradycardia during only the first ten seconds of the response and found it to be abolished by atropine or bilateral vagotomy. Our results are not incompatible with a sympathetic component in the steady state, as found by Daly & Scott (1962).

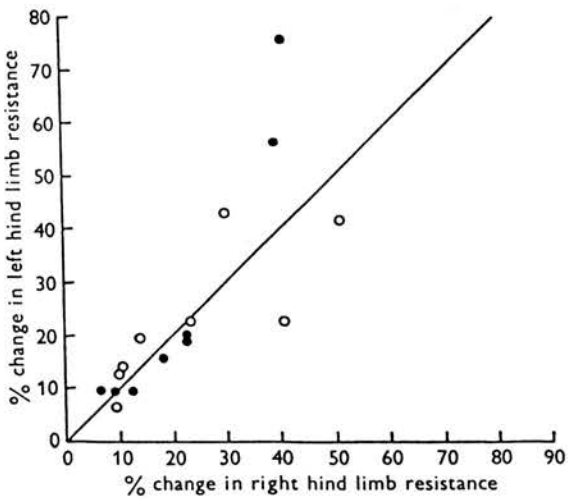

Fig. 4. Spot diagram of ipsilateral against contralateral responses: reflex vasoconstriction. Graphical representation of the results in Table 2. Open circles represent effects of stimulation of the right carotid body (RCB) and filled circles represent the effects of stimulation of the left carotid body (LCB). Percentage changes in right hind limb resistance are plotted on the abscissa and percentage changes in left hind limb resistance on the ordinate. The line of equality is indicated.

Reflex vasoconstriction. Our results confirm the findings of Daly & Scott (1962) that a primary reflex response to carotid body stimulation is vasoconstriction, mediated entirely by sympathetic adrenergic nerves.

It is evident from Fig. 4 that whichever carotid body we stimulated, we saw a reflex vasoconstriction in both hind limbs, and that the response on neither side was consistently larger than that on the other. This sympathetically mediated reflex thus differs in its pattern of distribution from the parasympathetically mediated bradycardia.

The only previous work of which we are aware on the distribution of a sympathetically mediated reflex from the carotid bodies is that of

Fedorchuk (1954), who found that the reflex release of catecholamines occurred solely or predominantly from the adrenal gland on the same side as a stimulated carotid body in the cat. McQueen & Ungar (1970) did not find such a predominance in the dog.

Reflex increase in phrenic activity. Our results, illustrated in Fig. 5, indicate that stimulation of either carotid body gives rise to uniform increases in activity in both phrenic nerves in the dog. Our two experiments in cats suggest that the pattern is similar.

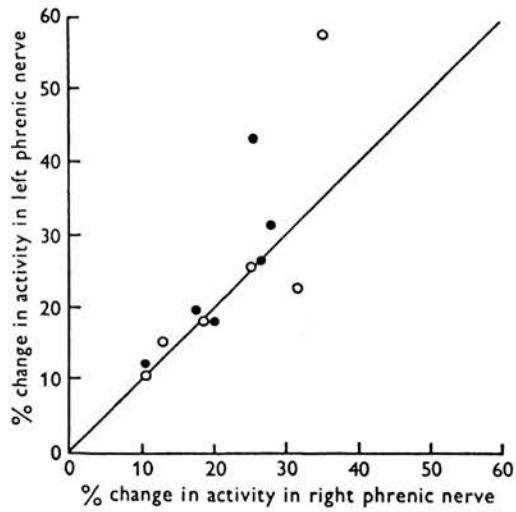

Fig. 5. Spot diagram of ipsilateral against contralateral responses: reflex increase in phrenic nerve activity. Open circles represent the effects of stimulation of the right carotid body (RCB), and closed circles the effects of stimulation of the left carotid body (LCB). Percentage changes in activity in the right phrenic nerve are plotted on the abscissa, and percentage changes in the left phrenic nerve on the ordinate. The line of equality is indicated.

We thus differ from Fedorchuk (1957) who found that after ablation of one carotid body, an intravenous injection of cytisine only increased activity in the phrenic nerve on the side of the intact carotid body. Having excluded the possibility of a species difference between dogs and cats, the possibility remains that denervation of one group of receptors may alter the pattern of responses to stimulation of the remaining receptors.

We conclude from our results that the central pathways from chemoreceptor afferent fibres to cardio-inhibitor fibres in the vagi must be different from their pathways both to the sympathetic fibres to the limbs and to phrenic motoneurones. Pathways to the first are predominantly ipsilateral, but with the last two there seems to be a balanced influence on the two sides.

We wish to thank Professor E. W. Horton for his valuable comments and suggestions. One of us (D.S.McQ.) was supported by a Medical Research Council Scholarship. Part of this work was carried out in the Department of Pharmacology, The School of Pharmacy, University of London. We thank Mr D. King for his technical assistance.

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Journal of Physiology, **202**, 30–31 *P*]

The direct and crossed vagal components of the reflex bradycardia following stimulation of the carotid body chemoreceptors in the dog

By D. S. McQueen* and A. Ungar. *Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Sq. London, W.C. 1*

Nazarenko (1958) found that the bradycardia following intravenous injection of potassium cyanide into cats with one carotid body extirpated could be abolished in two out of six animals by ipsilateral cervical vagotomy, and in eight out of fifteen by contralateral vagotomy.

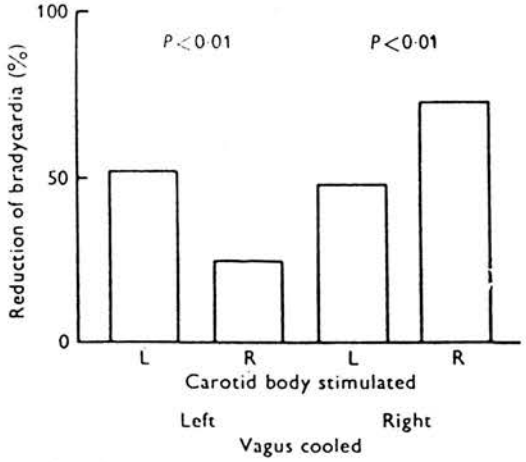

Fig. 1. The effect of cooling the right and left vagi on the reflex bradycardia from each carotid body in six dogs. For each vagus cooled, the difference between reduction of bradycardia from the left and right carotid bodies is statistically significant at the level of probability indicated.

We used dogs, pre-treated with morphine, under choralose–urethane anaesthesia, with lungs ventilated by a Starling 'Ideal' pump. The two carotid bodies were stimulated independently by infusion through the superior thyroid arteries, cannulated towards the heart, of 20–100 nM of suberyl dicholine over 15 sec. In some experiments sodium cyanide was used and gave similar results. The carotid bodies were stimulated in turn, before and after cooling each vagus nerve in the neck to $-2°$ C.

Our first results were irregular, vagal cooling giving sometimes an increase and sometimes a decrease in the bradycardia. Since the vagi carry

* M.R.C. Research Scholar. [P.T.O.

impulses from pulmonary receptors which modify the cardiac responses to chemoreceptor stimulation (Daly & Scott, 1963) we repeated our experiments in open chested dogs after cutting the pulmonary branches of the vagi so that complicating effects from the lungs could be excluded. The results now became consistent, vagal cooling giving a reduced bradycardia in forty out of forty-one tests in six dogs. The pooled results are represented in the histogram.

We conclude that when the chemoreceptors are stimulated, cardio-inhibitor activity in each vagus nerve is induced predominantly by the carotid body on the same side, but that there is a consistent component from the opposite side. This pattern is masked if the afferent pathways from pulmonary receptors are intact.

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Journal of Physiology, **203**, 48–49*P*

The direct and crossed components of the reflex vasoconstriction in the hind limbs of the dog following stimulation of the carotid body chemoreceptors

By D. S. McQueen* and A. Ungar. *Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London, W.C.* 1

Bernthal, Motley, Schwind & Weeks (1945) showed that the reflex vasoconstriction in the hind limb following carotid body chemoreceptor stimulation is due solely to increased sympathetic tone.

There is evidence that when the carotid body of one side is stimulated the resulting reflex bradycardia is mediated predominantly by the vagus nerve on the same side (Anichkov & Belen'Kii, 1963; McQueen & Ungar, 1969). Fedorchuk (1954) also found the reflex release of catecholamines from the adrenal medulla following stimulation of one carotid body to be predominantly or entirely ipsilateral.

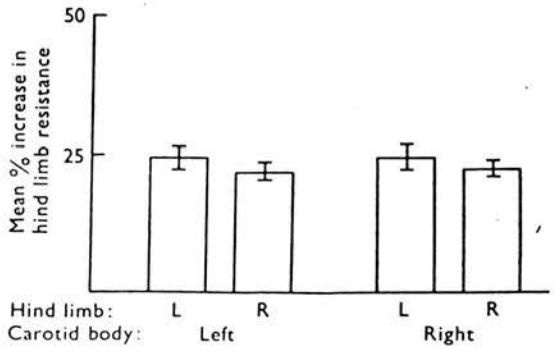

Fig. 1. The effects of stimulation of one carotid body on the vascular resistance of the separately perfused hind limbs. Pooled results of 29 tests in 7 dogs.

We have examined the sympathetically mediated increase in hind limb vascular resistance obtained on carotid body chemoreceptor stimulation and have found no evidence of ipsilateral predominance.

Dogs were pre-treated with morphine, anaesthetised with chloralose and urethane, and artificially ventilated by a Starling Ideal pump. Both vago-sympathetic trunks were cut in the neck. The hind limbs were separately perfused at constant flow with blood from the same animal. The two carotid bodies were independently stimulated by suberyl dicholine (McQueen & Ungar, 1969) and changes in the vascular resistances of the limb were determined.

* M.R.C. Research Scholar.

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Stimulation of either carotid body gave an increase in the vascular resistance of both hind limbs in each of eight dogs. The responses in both hind limbs when the carotid bodies were stimulated in turn are shown in Fig. 1. There are no significant differences between the responses.

There thus appear to be differences between the efferent pathways of reflexes from the carotid bodies such that sympathetically mediated vasoconstriction is equally distributed on the two sides, while parasympathetically mediated bradycardia is predominantly ipsilateral.

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Journal of Physiology, **204**, 131–132*P*

The direct and crossed components of the reflex increase in phrenic nerve activity following stimulation of the carotid body chemoreceptors in the dog

By D. S. McQueen* and A. Ungar. *Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Sq., London, W.C. 1*

Fedorchuk (1957), using decerebrate cats having the carotid body of one side denervated, injected chemoreceptor stimulant drugs intravenously. She found an increase in electrical activity in the phrenic nerve on the side of the intact carotid body and none on the side of the denervated carotid body.

We have compared the changes in electrical activity of both phrenic nerves following selective stimulation of the two carotid bodies in turn.

Dogs were premedicated with morphine and anaesthetized with α-chloralose and urethane; their lungs were artificially ventilated by a Starling Ideal pump. Both vagosympathetic trunks were cut in the neck in order to exclude secondary reflexes from pulmonary stretch receptors. Electrical activity in the central cut end of each phrenic nerve in the neck was integrated and recorded with an ultra-violet oscillograph. Each carotid body was independently stimulated by the method described by McQueen & Ungar (1969) with suberyl dicholine as stimulant.

Stimulation of either carotid body resulted in increased activity in both phrenic nerves. In sixty-nine tests on three dogs the mean differences between the increments in activity in the two phrenic nerves when one or other carotid body was stimulated were not statistically significant ($P > 0{\cdot}2$).

We conclude that there are both direct and crossed components in the reflex influence of the carotid body chemoreceptors upon the diaphragm of the dog, and that unilateral chemoreceptor stimulation does not disturb the balance of respiratory motor activity on the two sides.

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* M.R.C. Scholar.

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Journal of Physiology, **207**, 20–21 *P*

The direct and crossed components of the reflex release of catecholamines from the adrenal medulla of the dog following stimulation of the carotid body chemoreceptors

By D. S. McQueen*† and A. Ungar.* *Department of Pharmacology, School of Pharmacy, London University, Brunswick Square, London, W.C. 1*

Anichkov & Belen'kii (1963) state that in decerebrate cats, with one carotid sinus nerve cut and one adrenal gland removed, intravenous injections of nicotine produced a pressor effect if the intact sinus and intact adrenal gland were on the same side but not if they were on opposite sides of the body. This implies a predominantly ipsilateral reflex pathway from the carotid body to the adrenal medulla.

We have measured the separate changes in catecholamines released from the two adrenal medullae while selectively stimulating the two carotid bodies in turn.

Our experiments were performed on dogs, pre-treated with morphine, anaesthetized with α-chloralose and urethane and artificially ventilated.

Table 1. Output of catecholamines from both adrenal glands before (control) and during (test) unilateral carotid body chemoreceptor stimulation. Pooled results of thirty-two tests in seven dogs

		Noradrenaline		Adrenaline		
		Left adrenal gland	Right adrenal gland	Left adrenal gland	Right adrenal gland	
Left carotid body	Control	6·5	6·7	18·9	18·8	ng/kg min
	Test	11·4	14·0	41·7	41·7	ng/kg min
		$P > 0·2$		$P > 0·5$		
Right carotid body	Control	7·4	3·2	19·6	9·8	ng/kg min
	Test	21·8	13·4	74·8	39·9	ng/kg min
		$P > 0·3$		$P > 0·2$		

Both cervical vagosympathetic nerve trunks were cut and the animals were eviscerated. Adrenal venous blood was collected through cannulae in the lumbar veins, the adrenolumbar veins being occluded at their junctions with the vena cava. The concentrations of adrenaline and noradrenaline in the adrenal venous blood were estimated using a modification of the method of Häggendal (1963).

* Present address: Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh.
† M.R.C. Research Scholar.

[P.T.O.

Each carotid body was independently stimulated by infusion of suberyl dicholine di-iodide as described by McQueen & Ungar (1969). Samples for estimation of catecholamines were collected before, and during each stimulus.

For both adrenaline and noradrenaline there is no statistically significant difference between the amounts released from the two adrenal glands following stimulation of either carotid body.

There is a significantly greater amount of adrenaline released than of noradrenaline.

We conclude that there are equipotent direct and crossed components in the reflex influence of the carotid body chemoreceptor upon the adrenal medulla of the dog and that chemoreceptor stimulation releases both adrenaline and noradrenaline.

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Can drugs replace hypoxic drive in respiratory depression?

D. S. McQueen and A. Ungar*
Department of Pharmacology, University of Edinburgh

The respiratory control of cats deeply anaesthetized with pentobarbitone or with chloralose depends on hypoxic drive from the arterial chemoreceptors. Changing their inspired gas from air to pure oxygen will severely depress or even arrest the respiratory movements. We have compared the effectiveness of suberyl dicholine diiodide (SDC) with that of nikethamide in this situation. SDC stimulates the arterial chemoreceptors, and does not act centrally (Mikhel'son, Rybovlev, Gorelik & Dardymov, 1957).

Five cats were anaesthetized with chloralose. The anaesthetic solution was slowly infused intravenously until their P_{a,CO_2}, while breathing air, rose to between 55 and 60 torr (about twice the level for unanaesthetized cats). SDC (50 μg/min) and nikethamide (12·5 mg/min) were infused in turn for periods of 5 min, allowing 30 min for recovery after each drug infusion. The results are shown in Table 1.

TABLE 1. *Mean results from five cats (female, 2–3 kg) under chloralose*

	Breathing air			Breathing 100% oxygen		
	Control	SDC	Nikethamide	Control	SDC	Nikethamide
$\dot{V}$ ml/min	436 ± 74	577 ± 84	674 ± 95	273 ± 46	394 ± 46	376 ± 79
Tidal volume ml	53 ± 6	69 ± 6	47 ± 6	42 ± 8	51 ± 9	32 ± 5
Respiratory rate breaths/min	8·5 ± 1	8·5 ± 1	14·1 ± 1	7·4 ± 2	8·9 ± 2	12·9 ± 2
P_{a,CO_2} torr	58 ± 5	50 ± 6	49 ± 5	70 ± 6	52 ± 4	60 ± 5

SDC infused at 50 μg/min and nikethamide at 12·5 mg/minute.

The results were analysed by paired *t* tests ($P < 0{\cdot}01$ being taken as significant).

SDC increased ventilation ($\dot{V}$) by an effect on tidal volume, with no significant effect on respiratory rate. SDC was significantly more effective in lowering P_{a,CO_2} during oxygen breathing than during air breathing, although the increases in $\dot{V}$ were similar.

Nikethamide increased $\dot{V}$ by an effect on respiratory rate with no significant effect on tidal volume. Nikethamide was significantly less effective in increasing RMV during oxygen breathing than during air breathing.

For a given increase in $\dot{V}$, SDC was more effective than nikethamide in lowering P_{a,CO_2}. This would be expected of a drug acting on tidal volume rather than respiratory rate.

The response to SDC was sustained during infusions lasting 30 minutes.

We conclude that SDC may have advantages over centrally acting drugs for the replacement of hypoxic drive, making it possible for pure oxygen to be breathed in respiratory depression.

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Treatment of newborn rats with capsaicin reduces baro- and chemo-reflex activity

By Susan M. Bond, F. Cervero* and D. S. McQueen. *Departments of Pharmacology and * Physiology, University Medical School, Edinburgh EH8 9AG*

Substance P (SP)-like material has been found in the nucleus tractus solitarius where baroreceptor and chemoreceptor primary afferent fibres terminate, and it has been suggested that SP is a transmitter at the central nerve terminals of baro- and chemoreceptor afferent nerve fibres (Gillis, Helke, Hamilton, Norman & Jacobowitz, 1980). Treatment of newborn rats with capsaicin causes a degeneration of unmyelinated afferent neurones (Jancsó, Király & Jancsó-Gabor, 1977) and reduces immunoreactivity to various peptides in unmyelinated afferent fibres (Gamse, Holzer & Lembeck, 1980), so might be expected to reduce baro- and chemoreceptor reflex activity.

We have studied reflex blood pressure and respiratory changes (see McQueen, 1973) in response to either 60 sec bilateral carotid occlusion (BCO) or intravenous sodium cyanide in adult Sprague–Dawley rats anaesthetized with pentobarbitone (40 mg.kg^{-1} I.P.). The animals had been treated under halothane anaesthesia with a single injection of either capsaicin (50 mg.kg^{-1} S.C.) or drug vehicle (50% polyethylene glycol:50% saline) 2–4 days after birth. Mean B.P. rose in response to BCO by 24·9 ± 1·9 mmHg (mean ± S.E.) from an average pre-occlusion value of 114 ± 5·2 mmHg in ten vehicle-treated rats, but only by 14·1 ± 1·9 mmHg from 109 ± 10 mmHg in seven capsaicin-treated animals; the difference between mean pressor responses is statistically significant ($P < 0·01$, Wilcoxon two-sample test). Respiratory responses measured during the first 20 sec following cyanide injections (2·5–800 μg I.V.) were greater in control animals than in those which had received capasicin. For example, in vehicle-treated rats a near-peak response was obtained with 100 μg-NaCN, respiratory minute volume (RMV) increasing from a pre-injection level of 174 ± 17 ml. min^{-1} to 412 ± 64 ml. min^{-1} ($n = 8$), whereas in capsaicin-treated animals, whose mean body weight was not significantly different from that of the controls ($P > 0·05$), the same dose of cyanide increased RMV from 132 ± 21 to 235 ± 31 ml. min^{-1} ($n = 7$; $P < 0·05$ *vs.* vehicle-treated). P_{a,CO_2} averaged 4·0 kPa in controls and 4·5 kPa in capsaicin-treated rats ($n = 4$).

We can conclude that both baroreceptor and chemoreceptor reflex activity are significantly reduced in pentobarbitone anaesthetized adult rats which have been treated neonatally with capsaicin, and that this is likely to result from the destruction of unmyelinated baro- and chemoreceptor afferent fibres, although actions on the chemosensory mechanism in the carotid body cannot be excluded.

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INFLUENCE OF NEONATALLY ADMINISTERED CAPSAICIN ON BARORECEPTOR AND CHEMORECEPTOR REFLEXES IN THE ADULT RAT

SUSAN M. BOND, F. CERVERO* & D.S. McQUEEN

Departments of Pharmacology and Physiology*, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

1 Baroreceptor and chemoreceptor reflex activity was studied in anaesthetized adult rats which had been treated neonatally with a single injection of capsaicin (50 mg/kg s.c.).

2 Pressor responses to bilateral carotid artery occlusion were significantly lower in capsaicin-treated rats compared with vehicle-treated controls. Pressor responses to intravenously injected noradrenaline were similar in the two groups of rats.

3 Resting respiratory minute volume and tidal volume were lower in anaesthetized capsaicin-treated animals than in vehicle-treated controls, but there was no significant difference in respiratory frequency.

4 The increases in respiration evoked by intravenous administration of the peripheral arterial chemoreceptor stimulant, sodium cyanide, or by breathing a hypoxic gas mixture, were significantly lower in capsaicin-treated rats compared with the controls.

5 It is concluded that baroreceptor and chemoreceptor reflex activity are significantly reduced in anaesthetized adult rats which had been treated neonatally with capsaicin, and that this is likely to result from the destruction of unmyelinated baro- and chemoreceptor afferent fibres.

Introduction

Substance P (SP)-like material is present in the carotid bodies of cats (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Cuello & McQueen, 1980; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Wills, 1980) and rats (Jacobowitz & Helke, 1980), in nerve fibres of the rat carotid sinus and aortic arch (Helke, O'Donohue & Jacobowitz, 1980), and in the petrosal and nodose ganglia which contain the cell bodies of baroreceptor and chemoreceptor nerves (Lundberg, Hökfelt, Nilsson, Terenius, Rehfeld, Elde & Said, 1978; Gillis, Helke, Hamilton, Norman & Jacobowitz, 1980; Helke *et al.*, 1980). SP-like material has also been detected in the nucleus tractus solitarii (NTS) of the medulla oblongata (Cuello & Kanazawa, 1978; Ljungdahl, Hökfelt & Nilsson, 1978; Helke *et al.*, 1980; Gillis *et al.*, 1980), a region where many baro- and chemoreceptor primary afferent nerves terminate (Crill & Reis, 1968; Lipski, McAllen & Spyer, 1977; Palkovits & Záborszky, 1977). Intracranial sectioning of cranial nerves IX and X, which contain baro- and chemoreceptor fibres, causes a reduction of SP-like material in the NTS (Gillis *et al.*, 1980), and microinjection of SP into the intermediate parts of the NTS has been reported to evoke hypotension and bradycardia in rats and cats (Haeusler & Osterwalder, 1980a; but cf. Talman & Reis, 1981), a response which is very similar to that obtained by activating the baroreflex. These findings have led to the suggestion that SP is a neurotransmitter at the central terminals of baro- and chemoreceptor afferent nerve endings (Gillis *et al.*, 1980; Haeusler & Osterwalder, 1980b; Helke *et al.*, 1980).

Treatment of newborn rats with capsaicin causes a degeneration of unmyelinated primary afferent neurones (Jancsó, Király & Jancsó-Gabor, 1977; Nagy, Vincent, Staines, Fibiger, Reisine & Yamamura, 1980; Scadding, 1980) and a reduction of SP-like immunoreactivity in the relay nuclei of unmyelinated afferent fibres in the central nervous system (Nagy *et al.*, 1980; Gamse, Holzer & Lembeck, 1980; Nagy, Hunt, Iversen & Emson, 1981). The SP content of other nuclei within the CNS which do not receive an unmyelinated projection of peripheral origin is not affected in animals treated neonatally with capsaicin (Gamse *et al.*, 1980; Nagy *et al.*, 1980). These observations indicate that administration of capsaicin to neonates causes the degeneration of a substantial proportion of peripheral unmyelinated afferent fibres, including those containing SP.

Among the unmyelinated afferent fibres affected by neonatal treatment with capsaicin are those which

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terminate in the sensory nuclei of cranial nerves V, IX and X (Jancsó & Király, 1980). This raises the possibility that cardiovascular control may be impaired in animals treated at birth with capsaicin, so in the course of a study on the relevance of unmyelinated afferent fibres to the mechanisms of nociception (Cervero & McRitchie, 1981), we decided to test baroreceptor and chemoreceptor reflexes in rats which had been treated neonatally with capsaicin. The objectives were to analyse the relative contribution of unmyelinated afferent fibres to these reflexes and to obtain further evidence concerning the role of SP in these sensory pathways. A preliminary account of some of the results has been published (Bond, Cervero & McQueen, 1982).

Methods

Sprague Dawley rats were used in this study. When the animals were between two and four days old they were anaesthetized with halothane (1% in oxygen) and littermates received either a single subcutaneous injection of capsaicin (50 mg/kg) or drug vehicle (1:1 polyethylene glycol 200:0.9% w/v aqueous sodium chloride). Three to seven months later the rats were used for the study of baro- and chemoreceptor reflexes. Most of the animals were males, the average body weights being 469 ± 48 g (controls) and 460 ± 47 g (capsaicin-treated).

Surgical procedures and recording techniques

Animals were anaesthetized with pentobarbitone (40 mg/kg, i.p.) and the trachea was cannulated, as were a femoral artery and vein. At the end of several experiments a catheter was also inserted into the rostral end of a common carotid artery and was used for measuring the pressure in the carotid sinus during bilateral carotid artery occlusion (BCO). Blood pressure was measured via a pressure transducer and displayed on a chart recorder (Devices, M4). Respiration was measured with an integrating pneumotachograph, as previously described (McQueen, 1973). The animals breathed either room air, 100% O_2, or a hypoxic gas mixture (10% O_2:90% N_2). Blood samples were taken from the femoral artery for the measurement of arterial blood gas tensions and pH (Radiometer BMS3).

Barorecepter function test

Bilateral carotid occlusion Rats were anaesthetized with pentobarbitone and breathed room air spontaneously. Small artery clips were applied to both common carotid arteries low in the neck for 60 s, with at least 5 min between successive occlusions. Systolic, diastolic, and mean (diastolic + ⅓ pulse pressure) blood pressures were determined before bilateral carotid occlusion (BCO) and at the peak of the pressor response to BCO (Figure 1). In some experiments occlusion was maintained for 120 s, but the pressor responses were not significantly different from those obtained using the shorter period of BCO. Three occlusions were performed in each animal: before, 5 min and 30 min after a supplemental dose of anaesthetic (6 mg, i.v.) and the average rise in mean BP was calculated. The level of anaesthesia affected the basal pressure but had little influence on pressor responses in these animals. Some experiments were also performed in which a comparison was made between the pressor response observed during air-breathing with that obtained when the animal breathed 100% O_2.

Noradrenaline

The pressor responses to doses of noradrenaline injected intravenously were determined in anaesthetized rats.

Chemoreceptor function tests

Sodium cyanide The peripheral arterial chemoreceptors were activated by intravenous injection (0.1 ml + 0.2 ml wash over 2–4 s) of various doses of sodium cyanide in spontaneously breathing rats. At least 5 min was allowed to elapse between successive injections. The increase in respiratory volume during the 20 s period immediately following the injection was calculated and plotted against dose to provide a dose-response curve. Cyanide experiments were performed after testing the baroreceptors by BCO in order to minimize the total number of animals used.

Hypoxic gas After completion of the surgical procedures and before performing any other tests the animals were switched from breathing room air to breathing 10% O_2:90% N_2 for 4 min, and the reflex increase in respiratory minute volume (RMV) determined. An arterial blood sample was taken before and 3 min after changing to hypoxic gas. The level of anaesthesia (assessed qualitatively) was similar in the two groups of rats.

Drugs

The drugs used were capsaicin (8 methyl-N-vanillyl-6-nonenamide, Sigma), sodium cyanide (BDH) and (−)-noradrenaline bitartrate (Koch Light).

Statistical analysis

Differences between group means were compared by

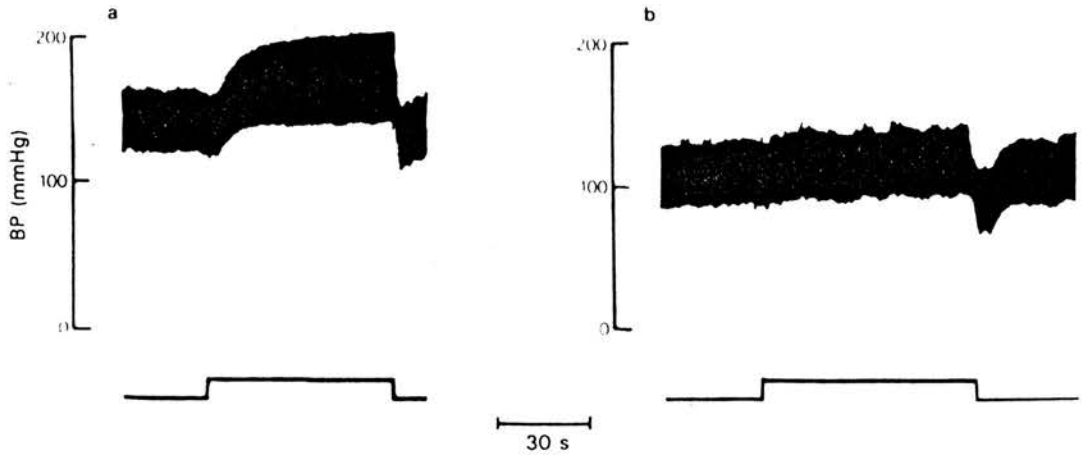

Figure 1 Pressor response to bilateral carotid occlusion (marker) in a control rat (a) compared with that obtained in a capsaicin-treated animal (b). Prolonging the period of occlusion did not significantly increase the response.

the Wilcoxon rank test, or Student's *t* test when there were insufficient data for the non-parametric test, and the null hypothesis rejected if $P < 0.05$.

Results

Baroreceptor function test

Bilateral carotid occlusion Reflex pressor responses to BCO (e.g. Figure 1) were determined in ten control (vehicle-treated) rats and seven which had been treated neonatally with capsaicin. Pre-occlusion mean BP was slightly lower in the capsaicin-treated anaesthetized animals breathing air than in the controls (see Figure 2a), but this difference was not statistically significant. During BCO the averaged reflex hypertension, whether expressed in terms of mean BP (Figure 2a), systolic BP or diastolic pressure was significantly lower ($P < 0.01$) in capsaicin-treated rats as compared with controls. On average the peak pressor response occurred 33.7 ± 9 s (s.e.mean) after applying the clips in control rats and 24.6 ± 7 s in capsaicin-treated animals ($P > 0.05$).

In seven control and four capsaicin-treated rats a comparison was made of the pressor response to BCO during air-breathing with that obtained in the same animal while breathing 100% O_2. The results are shown in Figure 2b. Basal mean BP was higher in controls breathing O_2 than when they breathed air, but the pressor responses were not significantly different. In the case of the capsaicin-treated animals basal blood pressure was higher during O_2-breathing, but the pressor responses were not significantly different from those obtained while breathing air ($P > 0.05$). On average the peak pressor response during O_2-breathing occurred 40.7 ± 8 s after applying the clips in controls and 31.7 ± 9 s in capsaicin-treated rats. The pressor responses in the controls were significantly greater than in the capsaicin-treated animals when breathing either air or oxygen ($P < 0.05$).

In two rats measurements of mean carotid sinus pressure showed that pressure fell on average to 32 mmHg immediately following BCO and recovered to 50 mmHg by 30 s, at which level it remained until 60 s when the clips were removed. Cutting the

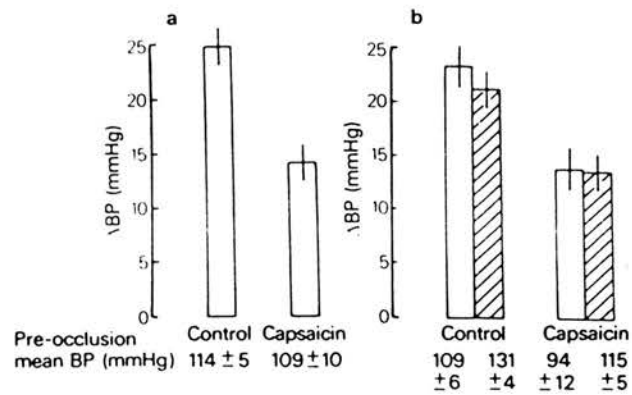

Figure 2 (a) The maximum rise in mean BP obtained during 60 s bilateral carotid occlusion (BCO) averaged from 10 control and 7 capsaicin-treated anaesthetized rats; vertical lines show s.e.mean. (b) In 7 controls and 4 capsaicin-treated animals the averaged pressor response to BCO during air-breathing (open columns) is compared to that obtained in the same animal during ventilation with 100% O_2 (hatched columns). The averaged mean BP ± s.e.mean measured just prior to BCO is given below the bar graphs.

carotid sinus nerves virtually abolished the reflex hypertension.

During the first 20 s of BCO there was a tendency for respiration to increase, particularly in control animals breathing air, but the rather variable nature of this response is reflected in the large standard errors (see Figure 3). The increase in respiration during BCO was smaller in capsaicin-treated rats and was abolished when these animals breathed 100% O_2, whereas in control rats the respiratory response to BCO was still present during O_2 breathing, although it was reduced.

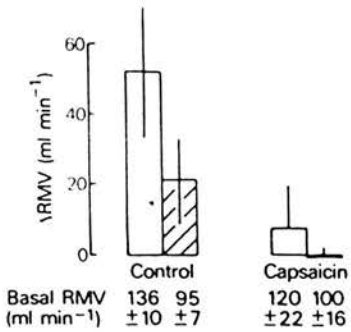

Figure 3 The averaged increase in respiratory minute volume (RMV, ±s.e.mean) observed during the first 20 s of a 60 s period of BCO during air breathing (open columns, data from 10 control and 7 capsaicin-treated rats) are shown. The hatched columns represent the respiratory change observed when 6 controls and 4 capsaicin-treated animals breathed 100% O_2. The averaged mean basal RMV ± s.e.mean measured just prior to BCO is given below the bar graphs.

Pressor responses to (−)-noradrenaline Noradrenaline was injected intravenously in two control and three capsaicin-treated rats and the averaged pressor responses evoked were plotted against the dose of noradrenaline. Results obtained are shown in Figure 4 from which it can be seen that although there may be differences between the two groups in the slopes of their dose-response lines, their pressor responses to noradrenaline (0.5–10 μg) were not significantly different ($P > 0.05$, 2-tailed t test, assuming normal distribution).

Chemoreceptor function tests

Sodium cyanide Respiratory responses to intravenous injection of various doses of the peripheral arterial chemoreceptor stimulant sodium cyanide (e.g. Figure 5) were studied in eight control and seven capsaicin-treated anaesthetized rats which were breathing air, and the results are summarized in Figure 6. Respiratory responses to cyanide were rather variable from animal to animal, as previously described (Colinet-Lagneaux, Troquet & Hermann-Gedang, 1966), and this is reflected in the rather high standard errors. The variability probably results from factors such as the level of anaesthesia, acid-base balance, and variation between individual animals in their responsiveness.

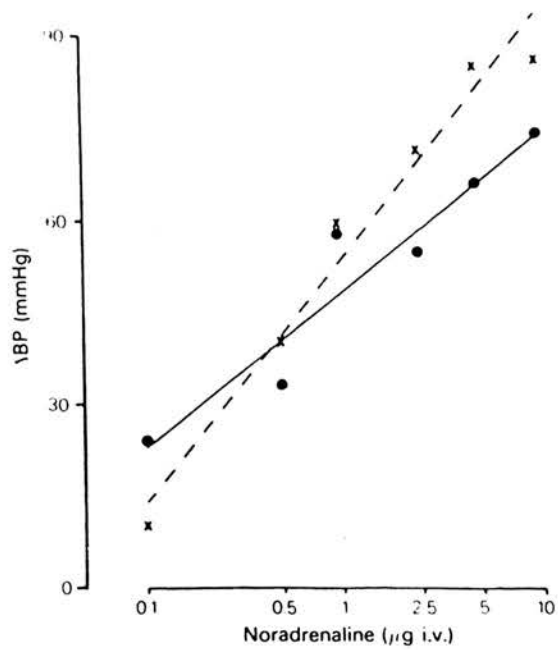

Figure 4 Averaged rise in mean BP in vehicle-treated controls (●——●, $n = 2$) and in capsaicin-treated rats (x---x, $n = 3$) plotted against dose of (−)-noradrenaline injected intravenously ($\log_{10}$ scale). Lines were fitted to the data by the method of least squares.
Mean BP measured immediately before injection of noradrenaline averaged 113 ± 4.5 mmHg in the control group and 103 ± 4 in the capsaicin-treated rats ($P > 0.05$).

Measurements made in the pre-cyanide control period showed that respiratory minute volume (RMV) and tidal volume (V_t) were significantly lower ($P < 0.01$) in the capsaicin-treated rats as compared with the vehicle-treated controls, but there was no significant difference in respiratory frequency (f) ($P > 0.05$). The mean basal values for capsaicin-treated rats (controls in brackets) were: RMV 112 ± 16 ml min^{-1} (156 ± 10); V_t 2.0 ± 0.2 (2.8 ± 0.1); f 56 ± 5 (56 ± 2) breaths min^{-1}. Control rats showed a significantly ($P < 0.05$) greater increase in RMV in response to injections of 10, 25, 50, 100 and 200 μg sodium cyanide (Figure 6). The low doses of cyanide (2.5–5 μg) had no appreciable effect on respiration in either group, whereas the very high, near-toxic, doses (400–800 μg) evoked re-

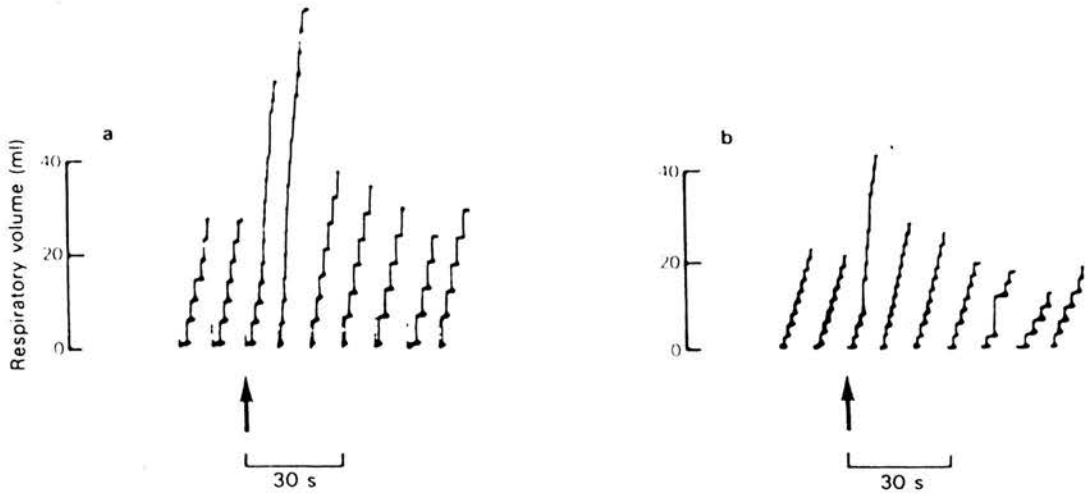

Figure 5 Respiratory response to an intravenous injection of 100 μg sodium cyanide at the arrow: (a) in a control, (b) in a capsaicin-treated rat. Each step in the ramped output from the pneumotachograph represents one breath, and the total height of each ramp is the respiratory volume in 10 s. Records read from left to right.

spiratory increases which, although greater in the control rats, were not significantly different from those obtained in capsaicin-treated animals; they caused substantial falls in BP. The increased RMV in both groups resulted from rises in f and V_t, but the latter was much more pronounced in control rats. In two control rats sodium cyanide (100 μg i.v.) evoked an increase in RMV within the first 20 s of injection which was abolished by cutting both carotid sinus nerves.

Hypoxia On switching to breathing the hypoxic gas mixture (10% O_2) an increase in respiration occurred in the two groups of rats, but both the absolute RMV and the increase in RMV were significantly greater in the controls (Table 1). Individual animals varied somewhat in their responsiveness and this is reflected in the high standard errors. The $PaCO_2$ measured during air-breathing was significantly higher in capsaicin-treated rats, as compared with the controls.

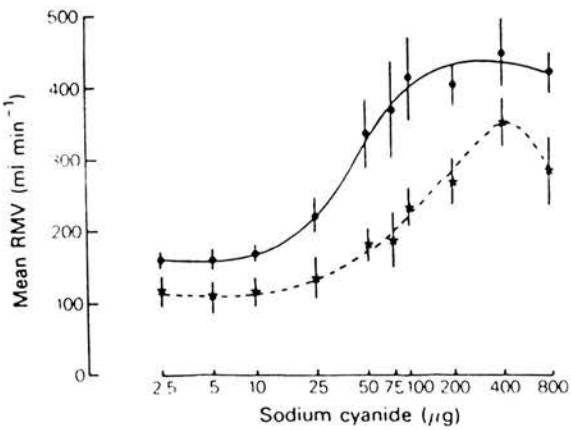

Figure 6 Pooled data from 8 control (●) and 7 capsaicin-treated rats (x) showing the mean respiratory minute volume (RMV) averaged over the first 20 s following intravenous injection of sodium cyanide (2.5–800 μg – $\log_{10}$ scale); vertical lines show s.e.mean. Lines were fitted to the data points by eye.

Discussion

Our results show that there is a significant reduction in baroreceptor and chemoreceptor reflex activity in adult rats which had been treated neonatally with capsaicin. It must be stressed that the experiments were performed under anaesthesia, so the effects we obtained may be peculiar to the anaesthetized animal. The contribution made by the anaesthetic agent could be determined by studying reflex activity in conscious capsaicin-treated rats.

Baroreceptor reflex

In a preliminary report Lorez, Haeusler & Aeppli (1981) state that treatment of neonatal rats with the same dose of capsaicin as we used did not change baroreceptor reflex function in adult animals. There was a reduction in the number of primary afferent SP-containing fibres in the rootlets of cranial nerves IX and X, but although the number of SP-containing fibres terminating in the nucleus tractus solitarius was markedly reduced in capsaicin-treated rats, SP-containing cells in this nucleus were unchanged. In the absence of more detailed information concerning

Table 1 Respiratory minute volume, arterial blood gas tensions, and pH measured in three control and three capsaicin-treated anaesthetized rats during air-breathing and again when breathing a hypoxic gas mixture (10% O_2)

		Control	*Capsaicin*
RMV (ml min^{-1})	Air	129 ± 27	93 ± 14
	10% O_2	270 ± 23	185 ± 23*
P_{aCO_2} (kPa)	Air	4.04 ± 0.12	4.67 ± 0.08*
	10% O_2	2.51 ± 0.26	3.03 ± 0.19
P_{aO_2}	Air	10.40 ± 0.40	9.83 ± 0.19
	10% O_2	5.56 ± 0.24	6.04 ± 0.32
pH	Air	7.44 ± 0.01	7.40 ± 0.03
	10% O_2	7.51 ± 0.01	7.51 ± 0.01

Respiratory volume was measured over a 10 s period starting at the peak of the respiratory response to hypoxia (49 ± 8 s after switching from air in controls, 53 ± 7 s in capsaicin-treated animals). Males were used for these tests (body weight of controls 350 ± 18 g; capsaicin-treated 342 ± 29 g). Values are expressed as means ± s.e.mean.
*$P < 0.05$ compared with vehicle-treated controls. 1 kPa = 7.5 mmHg

the experiments of Lorez *et al.*, we are unable to offer any explanation of why it is that we found a depression of baroreceptor reflex function in capsaicin-treated rats, whereas they did not.

The possibility exists that, during BCO, the fall in carotid sinus pressure might, by reducing carotid body blood flow, lead to stimulation of the carotid body chemoreceptors. This would be liable to cause reflex sympathetic vasoconstriction (McQueen & Ungar, 1971), and the pressor response to BCO might, therefore, be attributed partly to withdrawal of inhibitory baroreceptor activity and partly to chemoreceptor stimulation. Carotid chemoreceptor stimulation did seem to occur during BCO in control rats since respiration increased, albeit rather variably, when the animals were breathing air, but to a lesser extent when breathing 100% O_2, a condition in which chemoreceptor activity is greatly reduced. However, pressor responses to carotid occlusion during air-breathing were not significantly different from those obtained when on 100% O_2, and this finding suggests that the reflex rise in blood pressure was mainly due to withdrawal of baroreceptor tone. Chemoreceptor stimulation in spontaneously breathing animals is liable to activate the lung inflation reflex which can mask the primary chemoreceptor reflexes (Daly & Scott, 1962) and this might explain why the pressor response to BCO during air-breathing was no greater than obtained when the control animals breathed 100% O_2. The fact that capsaicin-treated animals showed a much smaller increase in RMV during BCO when breathing air and virtually none during O_2-breathing is consistent with a reduction in the sensitivity of their peripheral chemoreceptors.

Given that baroreflex activity was attenuated in capsaicin-treated rats, it might be considered surprising that basal BP was not elevated in these animals. However, it has to be noted that baroreflex activity had only been reduced, not abolished, and also that the animals were anaesthetized; anaesthesia can abolish the hypertension resulting from baroreceptor deafferentation in rats (De Jong & Palkovits, 1976). The similarity of control and capsaicin-treated rats in their pressor responses to noradrenaline suggests that the vascular component of the baroreceptor reflex arc was not appreciably affected by capsaicin.

Chemoreceptor reflex

Sodium cyanide is a classical stimulant of peripheral arterial chemoreceptors (see Heymans & Neil, 1958) and appears to have no direct effect on baroreceptors (McQueen, 1980a). Respiratory changes occurring soon after its intravenous administration can reasonably be attributed to stimulation of carotid body chemoreceptors since it has previously been shown, and confirmed in the present study, that the reflex increase in respiration obtained following the injection of cyanide in rats is abolished by destroying the carotid bodies or cutting the carotid sinus nerves (Colinet-Lagneaux *et al.*, 1966; Colinet-Lagneaux, Hermann-Gedang & Troquet, 1967; Sapru & Krieger, 1977).

The reflex respiratory response to intravenous sodium cyanide was significantly reduced in anaesthetized capsaicin-treated rats, and it was also found that RMV was lower and P_{aCO_2} higher than in the controls. Reflex hyperventilation in response to breathing 10% O_2 was not so pronounced in capsaicin-treated animals as in controls. However, these latter findings are difficult to interpret exclusively in terms

of reduced responsiveness to arterial chemoreceptor stimulation because in addition to stimulating peripheral chemoreceptors, systemic hypoxia may depress the CNS by a direct action. Also, reflex hyperpnoea causes a fall in P_{aCO_2} and rise in pH which will tend to remove central respiratory drive, and the changes in blood pressure that accompany hypoxia will also indirectly influence respiration. Nevertheless, the evidence from these respiratory studies can be taken as showing that the sensitivity of the peripheral chemoreceptors and/or the central components of the reflex had been altered by capsaicin.

Substance P and unmyelinated fibres

Neonatal administration of capsaicin causes a substantial reduction in the number of unmyelinated afferent fibres, including those that originate from internal organs and terminate in the brain stem nuclei where baro- and chemoreceptor afferent fibres project (see Introduction). In preliminary studies we have found that the population of unmyelinated fibres in the carotid sinus nerves of capsaicin-treated rats is severely reduced (Cervero & McQueen, unpublished observations). Small myelinated fibres can also be affected by capsaicin at the dose used in the present study, but to a lesser extent (Nagy *et al.*, 1981). However, unmyelinated efferent fibres do not seem to be affected by neonatal capsaicin (Cervero & McRitchie, 1982), so we can therefore conclude that the changes in baroreceptor and chemoreceptor reflex function observed in the present experiments most probably result from the loss of unmyelinated afferent fibres.

SP-like material is associated with unmyelinated primary afferent fibres, including those in cranial nerves IX and X, since capsaicin treatment leads to a substantial reduction in the SP content of these fibres. Our results are not incompatible with SP having a role in the baro- and chemoreflexes by being released at the central endings of unmyelinated primary afferent fibres in the brain stem, or possibly by influencing events at the peripheral sensory endings (see McQueen, 1980b). However, it is not possible to interpret the findings in terms of a reduction in SP because neonatal capsaicin does not specifically affect SP-containing fibres; there is evidence to suggest that it can affect the levels of other peptides (e.g. somatostatin in dorsal root ganglia: Kessler & Black 1981) as well as SP. Further studies are needed to establish whether the changes in reflex activity we have observed are due to changes in SP alone, to changes in other substances, or to a combination of factors.

Treatment of neonatal animals with capsaicin may offer a preparation in which the relative contribution of myelinated and unmyelinated nerve fibres to baro- and chemoreceptor reflexes can be studied and the central interaction between the two types of fibre analyzed.

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SECTION 2

ACETYLCHOLINE AND DOPAMINE

PAPERS 9 - 23

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Effects of Temperature on Carotid Chemoreceptor and Baroreceptor Activity

D. S. McQUEEN AND C. EYZAGUIRRE

Department of Physiology, University of Utah College of Medicine, Salt Lake City, Utah 84132

THE OBJECT OF the present work was to investigate the influence of local temperature changes on carotid chemoreceptor and baroreceptor nerve discharges with the aim of obtaining some insight into the mechanism of impulse initiation in these receptors.

Ishiko and Loewenstein (12) have shown that temperature changes have a profound influence on the magnitude of the generator potential of Pacinian corpuscles; also, it is known that temperature is a potent modulator of chemoreceptor activity (1, 9, 20). However, the latter studies suffer from a lack of quantitative treatment. Therefore, we felt that it was important to conduct a quantitative study on the effects of temperature on the chemo- and baroreceptor discharges, discharge being taken as a reliable index of receptor activity.

The results are discussed in terms of information they provide on chemoreceptor mechanisms; they are necessarily indirect, since we measured only carotid nerve activity and no attempts were made to record events occurring at the receptor level, such as changes in potential of nerve endings or glomus cells. The responses evoked by asphyxia, ACh, and NaCN at different temperatures are discussed.

METHODS

Cats of either sex weighing between 1.8 and 3.0 kg were anesthetized with sodium pentobarbital (Diabutal, 40 mg/kg ip). A cannula was inserted into the trachea low in the neck and connected to a demand-valve respirator (Ensco), spontaneous respiration being abolished by gallamine triethiodide (Flaxedil, 2 mg/kg iv). The animal was ventilated with air and the end-tidal CO_2 was continually monitored by an infrared CO_2 analyzer (Beckman), the P_{CO_2} being maintained at 20–25 mm Hg (atmospheric pressure being 638–650 mm Hg) by adjusting the frequency and volume of respiration. A femoral artery was cannulated and connected to a pressure transducer, a record of arterial blood pressure being obtained on a Grass polygraph and on magnetic tape (frequency response: DC to 2,500 Hz). A femoral vein was cannulated and used for drug administration. The rectal temperature of the animal was monitored and maintained at 37°C.

Alteration of carotid blood temperature

A common carotid artery was cannulated both ways (Fig. 1) and blood pumped from the animal at constant flow through siliconized rubber tubing by a roller pump (Cole Parmer). Before the blood was returned to the carotid bifurcation it passed through a 16-cm spiral of polyethylene tubing (240) positioned in close contact with a thermoelectric module (Borg-Warner Thermoelectric), heat exchange being assisted by coating the heat-exchange surfaces with thermal compound. A thermistor was positioned on the surface of the polyethylene tubing in the heat exchanger (H) and connected to a servo control system (S), which allowed the temperature of the blood circulating through the heat exchanger to be increased or decreased by controlled steps. The temperature of the blood at the carotid bifurcation was measured by a thermistor (T) probe (Yellow Springs Instrument Co., time constant 0.1 s) inserted via the external carotid artery, the output from the thermistor being obtained on the pen recorder and on tape. The sinus perfusion pressure was monitored (P) and maintained between 130 and 160 mm Hg, which allowed a flow rate of 1–3 ml/min, depending on the particular animal. All the tubing was siliconized and heparin (150 U/kg iv) was administered initially and every 4 h thereafter.

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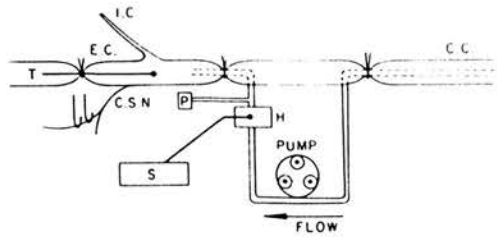

FIG. 1. Carotid perfusion system. Blood was pumped from the common carotid artery (CC) through a heat exchanger (H) and back to the artery about 2 cm below the carotid bifurcation. A thermistor bead mounted in H was connected to a servo-control system (S) which allowed the temperature in H, and hence that of the carotid blood, to be varied by controlled steps. Sinus perfusion pressure was monitored (P) and the temperature of the blood at the carotid bifurcation was measured by a thermistor probe (T) inserted via the external carotid artery (EC). With the exception of the lingual and external carotid arteries the blood vessels of the sinus region were not ligated (IC = internal carotid artery).

Recording of carotid sinus nerve activity

The carotid nerve on the same side as the cannulated carotid artery was dissected from surrounding tissues under a binocular microscope. Unless otherwise indicated, the ganglioglomerular nerves (connecting the superior cervical ganglion with the carotid body-sinus region) were cut. Exposed tissues were covered with warm mineral oil. The carotid nerve was sectioned centrally and fine strands were dissected from the nerve trunk. Recordings of multiple or single baroreceptor or chemoreceptor units were obtained by using bipolar platinum-iridium electrodes. Sensory nerve discharges were amplified, displayed on an oscilloscope, and recorded on tape. The output of the tape channel containing the action potentials was fed to a pulse-height discriminator and a counter-timer coupled with a digital-analog converter, the output from which was obtained on the pen recorder. In addition, the output from the pulse-height discriminator was fed to an analog-digital converter and spike frequency was computed (PDP-9). The analog data stored on magnetic tape were later played back through the oscilloscope and photographed.

When multiple units (generally two to five) were recorded, care was taken to ensure that additional units were not recruited during the course of temperature or drug-induced changes. If recruitment did occur the nerve filament was divided until only single or few active units were present and no recruitment occurred.

Chemoreceptor stimulants

The effect of chemoreceptor stimulants on discharge frequency was investigated by injecting a small volume of the stimulant solution (0.1 ml over 1–5 s) into the tubing before the roller pump, against the direction of flow. Injections were made every 5 min, 10 min after the temperature had been set at a new level. ACh chloride (Sigma) and NaCN (Merck) were studied, both substances being prepared in modified Locke solution: (NaCl 6.0 g, KCl 0.42 g, $CaCl_2$ 0.24 g, Trizma base 6.0 g, normal HCl 39 ml, distilled water to 1 liter, pH 7.41 at 37°C). Doses referred to are those of the salts.

The Pco_2 was varied by altering the volume of air delivered by the respirator until the desired end-tidal CO_2 was attained.

Data analysis

Chemoreceptor units were identified by their aperiodic pattern of discharge (2, 8) and their increase in discharge frequency when NaCN (5 μg) was injected into the carotid perfusion system. Baroreceptors were identified by their synchronous discharge with the pressure oscillations generated by the roller pump. Stopping the pump abolished or markedly reduced the discharge frequency, and NaCN had no effect on the activity of these units.

For both types of receptors, the discharge of either single or multiple units was analyzed over 30-s intervals, starting with a control period and then continuing after the onset of temperature changes for 2–4 min. Data were obtained as the average discharge over the 30-s period, as the maximum count in 1 s within this interval, and also as the total count for the 30-s period. In general, results from temperature changes are expressed as the discharge averaged over 30 s, starting 30 s after the temperature had reached the new level.

When the rate of change of temperature was studied, the temperature change was compared with the alteration in discharge frequency, this being performed directly from the data by computer. For drug studies, the increase in discharge elicited by chemoreceptor stimulants was plotted against time (time from onset of increase until return to control level) and both the average and the total counts were computed.

RESULTS

General temperature effects

Recordings obtained from either single or multiple chemoreceptor or baroreceptor units demonstrated that temperature

changes affected the spontaneous afferent activity. Raising the temperature increased the discharge, while lowering the temperature reduced it (Fig. 2).

The chemoreceptor response to a sustained temperature change was investigated in several animals. The carotid blood temperature alteration was effected over 20–30 s and a new level of chemoreceptor activity established about 60 s after onset of the temperature change. Increasing the temperature to 42.5°C and maintaining it at this level for 20 min elicited an increase in discharge, which remained reasonably steady for 5–10 min and then declined slowly, such that by 20 min the discharge was 50% of that obtained at peak activity; however, it was still 50% greater than the control (37°C) discharge. Eyzaguirre and Lewin (9) noted a similar pattern of response in vitro and suggested that adaptation or exhaustion of the receptors may have accounted for the decline in activity. In the present experiments we found no decrease in the ability of acetylcholine to elicit a response during the decline in spontaneous discharge at high temperature. This observation may indicate that adaptation of the discharge took place during exposure of the receptors to a constant temperature level. In fact, in partially adapted mechanoreceptors the discharge can be brought back to its original level if an additional stimulus is applied during the declining phase of discharge frequency (7). It is possible that in the case of chemoreceptors, the effects induced by temperature and ACh on discharge frequency may be additive.

Lowering the temperature to 20°C resulted in a diminished discharge which was maintained at a fairly constant value for the duration of the cooling period (15 min). The discharge returned to control values after bringing the temperature back to 37°C, this being the case for both the warming and cooling procedures.

The data obtained showed that a new steady state of spontaneous chemoreceptor activity was attained about 60 s after the onset of temperature change, and that temperature could be maintained at either low or high values for about 10 min without much change in the discharge. The temperature effects were reversible, as shown by the return to control discharge when the temperature was reset to 37°C.

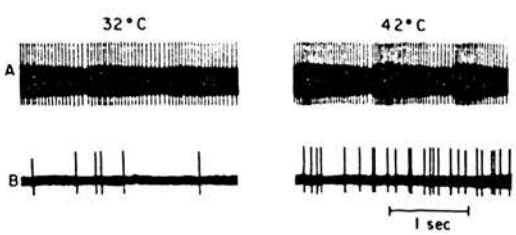

FIG. 2. Recordings of spontaneous activity from single baroreceptor (*A*) and chemoreceptor (*B*) units, 40 s after the carotid blood temperature had reached either 32 or 42°C.

Cycled temperature changes

Although the chemoreceptor discharge was capable of returning to control values after prolonged temperature changes, this was investigated at only three temperatures. Therefore, we decided to perform a more detailed study on the response to increasing the temperature (by 2° steps over 20–30 s) every 2 min from 37 to 42°C, thence to 26°C, and finally back to 37°C. The discharge was averaged over 30 s, 60 s after onset of the temperature change. Data from 11 recordings of single and multiple chemoreceptor units showed no apparent hysteresis in the temperature-response plot. There was a tendency for the discharge to reach a maximal value at about 42°C, further increase in temperature often resulting in a discharge which was less than that obtained at 42°C. In two units, the discharge did not return to control (37°) values after exposure to 26°C; another unit ceased firing at temperatures above 39°C. The reasons for this reduced activity were not investigated.

Data obtained during the cycled temperature changes were pooled and are shown in Fig. 3. Consideration of the Arrhenius plot (see later) suggested that two lines could be fitted to the data, one for values above 32°C, the other for values below 32°C. This "break" in the plot at 32°C was subjective but the observation that the slope of the line fitting the data below 32°C differed from the slope of the line above 32°C suggests a change in the relative importance of the rate-limiting reactions at about 32°C (14).

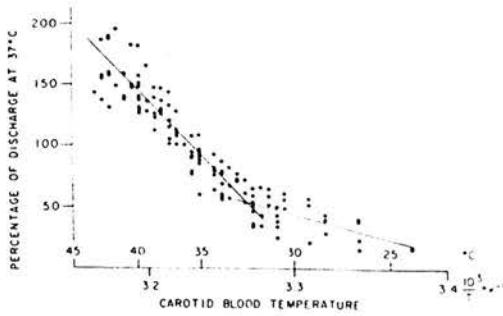

FIG. 3. Pooled data from eight recordings in seven cats in which the carotid blood temperature was varied within the range 23–44°C. The spontaneous chemoreceptor discharge obtained at a particular temperature is expressed as percentage of the discharge obtained from the same unit(s) at 37°C. Lines were fitted by the method of least squares using data obtained above 32°C for one line and data obtained below 32°C for the other.

For a 5° change of temperature at either side of normal carotid blood temperature (36–37°C) a 50% alteration of spontaneous chemoreceptor discharge was obtained.

Ramped temperature changes

In some chemoreceptor units a rapid temperature change (effected over 15–30 s) elicited a discharge which was rapid in onset, but which having reached a peak value declined to a new steady-state level (Fig. 4). This type of response was seen also in animals with intact ganglioglomerular nerves. The chemoreceptor response to a rapidly applied temperature change thus appeared to be divisible into a dynamic and a static component. In order to study further these two components, a ramp generator was used to increment the temperature by fixed amounts over different time intervals. The data obtained (Fig. 5) show a linear relationship between the rate at which the temperature is changed and the rate at which the chemoreceptor discharge alters, this applying both to increases and decreases in temperature. The chemoreceptors thus seem to be capable of making rapid responses and during the ramp period do not show much adaptation, at least not during the periods studied.

Only the rapidly applied temperature increase caused an overshoot of the chemoreceptor discharge, this also being observed in one of seven baroreceptor units investigated. A small undershoot in chemoreceptor activity was sometimes, although not invariably, seen when temperature was decreased. The pattern of response seemed analogous to that of muscle spindles where a dynamic and static component are obtained in response to stretch (15).

An overshoot in chemoreceptor discharge is also observed when a stimulus of hypercapnic blood is suddenly applied at the carotid chemoreceptors (3, 17). We did not investigate whether CO_2 changes were responsible for the present observations (see DISCUSSION).

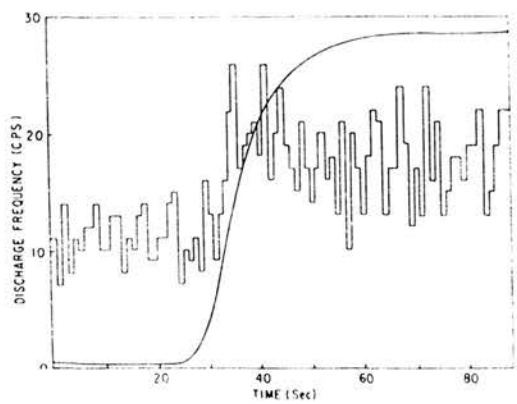

FIG. 4. Response of single chemoreceptor unit to a 6° increase in temperature (36–42°C, continuous line). This is typical of the response observed, namely a fast response with an overshoot (dynamic component) followed by stabilization at an intermediate discharge frequency (static component).

Temperature coefficient (Q_{10})

The factor by which rate constants differ, for a temperature increment of 10°C, is

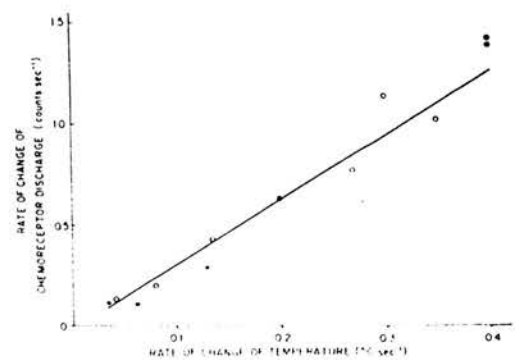

FIG. 5. Chemoreceptor units. The temperature was increased by 6° from 36°C (●) or decreased by 6° from 42°C (○) at different rates.

termed Q_{10} (the temperature coefficient): Q_{10} = discharge frequency at $(t + 10)$°C/ discharge frequency at t°C.

Q_{10} values for the chemoreceptors were obtained by recording the activity of either single or multiple units and varying the temperature such that steady-state values (60 s after onset of the temperature change) were obtained at about six temperatures in the range 26–44°C. It was found for most units that a plot of $\log_{10}$ discharge (counts/s) against temperature resulted in a linear relationship in the range 32–42°C and the Q_{10} value was calculated from the discharge frequencies at 32 and 42°C. Since we were not able to measure the temperature within the carotid body the assumption was made that the carotid blood temperature would be directly proportional to the carotid body temperature.

The activity of baroreceptor units was influenced by temperature (Fig. 2). As with the chemoreceptors, the discharge was averaged over 30 s, 60 s after onset of the temperature change, and at the same perfusion pressure. Most of the experiments were performed with a perfusion pressure of 140 mm Hg, although one was performed with a pressure of 210 mm Hg (maximal stimulus). The nerve discharge at 32°C was compared with that obtained at 42°C and the Q_{10} calculated.

Data obtained from chemoreceptors and baroreceptors are summarized in Table 1. Data were obtained also from two animals in which the ganglioglomerular nerves were not cut; chemoreceptor Q_{10} values of 3.03 and 2.12 were obtained, these being similar to values obtained with the nerves sectioned. In vitro experiments with superfused (Locke solution) cat carotid bodies gave Q_{10} values similar to those obtained in vivo (unpublished observations), suggesting that the high Q_{10} does not result from an effect of temperature changes on the blood or blood vessels (e.g., viscosity, liberation of vasoactive agents, etc.).

The difference between the baroreceptor and chemoreceptor Q_{10} values implies that the energy of activation of the rate-limiting step for the two types of receptors is different.

Apparent activation energy (μ)

Although the Q_{10} provides a measure of the influence of temperature on a biological process and is widely used, it is merely an empirical description without theoretical implications or justifications. A more sophisticated method of expressing quantitatively the effects of temperature on biological systems is to apply the Arrhenius equation (10, 13, 14).

$$k = Ae^{-\mu/RT}$$

where k is the velocity constant for the particular reaction, A is a constant, μ is a constant referred to as the apparent (experimental) activation energy, R is the gas constant, and T the temperature in degrees absolute. A plot of $\ln k$ against $1/T$ should give a straight line with a slope of $-\mu/R$.

The data obtained from experiments in which spontaneous chemoreceptor activity was examined at different temperatures were plotted according to the Arrhenius equation and a linear relation between the logarithm of the discharge frequency and the reciprocal of the absolute temperature was obtained (see Figs. 3 and 6). The significance of the μ value will be discussed later in relation to results obtained with ACh.

Temperature and gas-tension changes

In five cats the end-tidal CO_2 was raised by altering the stroke volume of the respirator. Although the gas-tension changes are expressed in terms of end-tidal CO_2, reducing the stroke volume of the pump would also affect the oxygen tension of the blood. The stimulus is thus an asphyxic one rather than a purely hypercapnic one.

TABLE 1. *Temperature coefficient Q_{10} determined between 32 and 42°C*

Type of Unit	No. of Recordings	No. of Animals	Q_{10} (Avg)	SEM	Range
Baroreceptor	8	5	1.27	0.06	1.08–1.50
Chemoreceptor	16	9	2.96	0.18	1.70–4.25

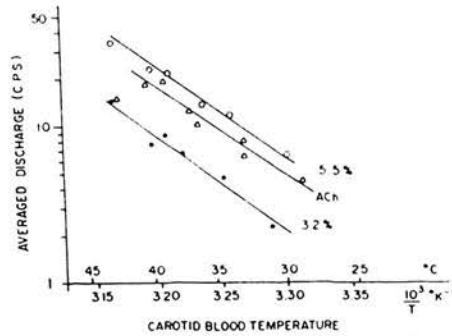

FIG. 6. Response of a single chemoreceptor unit ($\log_{10}$ scale) to temperature changes at end-tidal CO_2 levels of 3.2% (●) and 5.5% (○). The discharge is averaged over 30 s, 60 s after the temperature change. The value of μ for both levels is 27 kcal. Also shown is the response of another single chemoreceptor unit to injections of ACh (10 μg) at various temperatures (Δ). The averaged discharge is shown and the μ value for data between 29 and 42°C is 26 kcal. A similar μ value is obtained if total counts are plotted rather than averaged discharge.

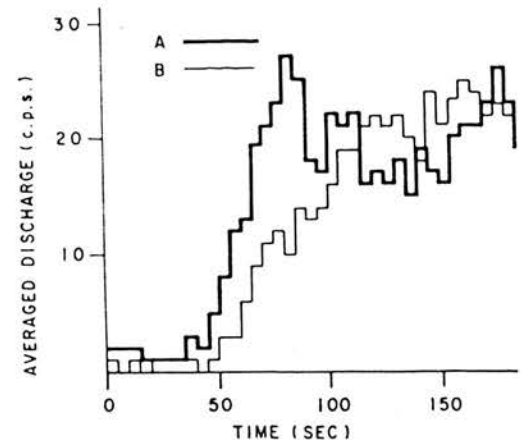

FIG. 7. Spontaneous chemoreceptor discharge (two units), averaged over 5-s intervals, against time after stopping the respirator (at 0 s). For A the carotid blood temperature was maintained at 37°C, and for B it was 24°C. It took about 25 s for blood to pass through the carotid perfusion system. There is a dynamic component in the response at 37°C which is not seen at 24°C.

The influence of temperature on spontaneous chemoreceptor activity at different end-tidal CO_2 levels is illustrated by Fig. 6. The data show that temperature has similar effects at the two CO_2 levels (Q_{10} and μ values similar at the two levels). This implies that the energy of activation of the rate-limiting reaction(s) is not affected by raising the CO_2 tension (and probably lowering the O_2 tension) at the chemoreceptors.

In one experiment air was replaced by 100% O_2 as the ventilating gas. The Q_{10} for the chemoreceptor discharge was unaltered in both the control state (CO_2 3.2%) and with a hypercapnic stimulus (CO_2 5.5%) as compared with the discharge obtained on air. The discharge was decreased at all temperatures studied (26–42°C) as compared with the discharge obtained when air was used as the ventilating gas.

In two cats (Fig. 7), recordings of chemoreceptor activity were obtained while the respirator was stopped, the sinus region being perfused at constant flow with blood at either 24 or 37°C. At 37°C a brisk increase in chemoreceptor discharge occurred after stopping the respirator (time lag in the sinus perfusion circuit being about 25 s) and a new level of discharge was established which persisted for the duration of asphyxia, about 4 min. When the carotid blood was cooled to 24°C, activity in the same filament took longer to develop although it eventually reached the same level of activity seen at 37°C. The discharge at 24°C did not show the overshoot seen at 37°C, but it persisted for the duration of asphyxia.

This observation demonstrates that at 24°C, the system is capable of producing a maximal response (similar in magnitude to that obtained at 37°C), although the dynamic component of the response does not occur at low temperatures.

Temperature and drug effects

ACh and NaCN were investigated for their ability to elicit a chemoreceptor discharge at various carotid blood temperatures. The procedure was to inject doses of the stimulants (ACh 6–100 μg; NaCN 0.5–20 μg) into the carotid perfusion system at blood temperatures of 24, 32, 37, and 42°C.

Dose-response data were obtained at the different temperatures and were expressed as: averaged discharge at x°C (where x = 24, 32, or 42°C)/averaged discharge at 37°C elicited by the same dose of stimulant. Values were also obtained using total counts rather than the averaged counts (see METHODS).

The ratios obtained from different units in different animals were pooled and are shown in Fig. 8, from which it can be seen that the response to both ACh and NaCN is affected by temperature and that although total counts and averaged counts show a similar trend, there are differences between the parameters at certain temperatures. At 24°C, both stimulants evoke a reduced discharge as compared with that obtained at 37°C, but there is still a marked stimulant effect. The stimulants differed in that although both acted equally well over a longer period at reduced temperatures (as compared to the effects at 37°C), NaCN took much longer to act than did ACh. This aspect of the results will be examined in detail in a subsequent publication.

An Arrhenius plot of chemoreceptor discharge elicited by 10 μg ACh at various temperatures was made (Fig. 6). The μ value calculated from the slope of the line was very similar (within experimental error) to that obtained with spontaneous activity or hypercapnia.

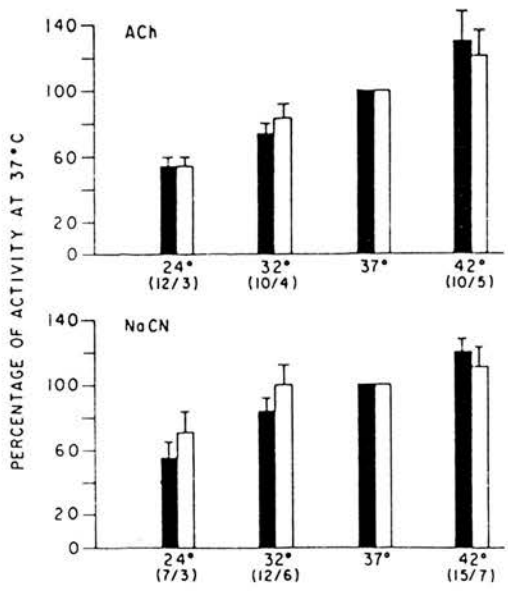

FIG. 8. Pooled data obtained from chemoreceptor units in response to injections of acetylcholine or sodium cyanide at different carotid blood temperatures. For a particular dose of stimulant the discharge elicited by 24, 32, or 42°C is expressed as a percentage of the discharge evoked at 37°C. Data are expressed as the mean ± SEM, shaded rectangles representing the averaged discharge, open rectangles the total discharge. Figures in parentheses are the number of recordings per animals studied.

DISCUSSION

The results show that chemoreceptor activity is increased by raising the carotid blood temperature and decreased by lowering it, these changes in spontaneous discharge being readily reversed within the temperature range studied. The findings confirm earlier observations on the effects of temperature on chemoreceptor activity (see INTRODUCTION). Baroreceptors respond to temperature changes in a similar manner, but the change in discharge frequency is not so marked.

Spontaneous chemoreceptor activity

In the majority of experiments, the ganglioglomerular nerves were cut to ensure that changes in carotid blood temperature did not affect the chemoreceptor discharge reflexly via the sympathetic nervous system. However, experiments in which the ganglioglomerular nerves were left intact showed similar results in terms of Q_{10} values, discharge overshoot, and drug responses.

A rapidly applied temperature change would often evoke a chemoreceptor discharge with an overshoot or dynamic component. A similar dynamic component was seen when the chemoreceptors were exposed to an asphyxic stimulus at 37°C. The mechanism of the dynamic component was not investigated.

The Q_{10} determined in the steady state was 2.96 for chemoreceptors and 1.27 for baroreceptors in the range 32–42°C. Our Q_{10} value for carotid chemoreceptors is thus similar to the value of 2.5 obtained by Paintal (20) for the cat aortic chemoreceptors. The observation that chemoreceptors have a high Q_{10} is suggestive that a chemical process is involved in the rate-limiting reaction, while the low Q_{10} for baroreceptors suggests that a physical process is involved in the rate-limiting step. However, there are problems associated with the use of Q_{10} values to establish that a particular reaction is either physical or chemical in nature (see ref 10, 11, 21). What we can conclude from our data is that the energies of activation of the rate-limiting reactions

for the two types of receptors differ, this being in contrast to Paintal's (20) conclusion that aortic chemoreceptors have Q_{10} values "similar to mechanoreceptors in general." Furthermore, there was a linear relationship between the logarithm of the chemoreceptor discharge and the reciprocal of the absolute temperature over the range 32–42°C, thus conforming with the Arrhenius equation and allowing calculation of μ. The high μ values obtained (Fig. 6) provide strong evidence that a chemical reaction is involved in the rate-limiting reaction at the chemoreceptors.

Chemoreceptor stimulation

Asphyxia at 37°C resulted in an increased chemoreceptor discharge which was more rapid in onset than the discharge elicited by asphyxia at 24°C. The maximal level of activity attained at the two temperatures was similar and demonstrates that at 24°C, the ability of the nervous mechanism to conduct discharges had not been significantly affected. The slower rate of change of discharge at lower temperatures in response to chemoreceptor stimulation has been described previously (18, 20). The present findings show that although the metabolic process is slowed by the temperature reduction, eventually the process is activated to about the same extent as at 37°C. However, besides changes in metabolic activity, reduced temperature may influence the sensitivity of the receptor region (12, 19, 20), cholinesterase activity (5), blood gas tensions and pH, blood viscosity, and probably other factors that may modify the nerve discharge elicited in response to a given stimulus. For instance, blood flow through the carotid body may be affected by temperature due to either constriction or dilatation of the intraglomerular vessels. These variations in blood flow may be either total or regional; the latter may be produced by internal redistribution of blood if the carotid body a-v shunt changes its lumen with temperature variations. Changes in all these factors, evoked by different temperatures, make it difficult to ascribe Q_{10} changes exclusively to receptor mechanisms. For instance, changes in blood pH and/or gas tensions may vary the intensity of the applied stimulus (asphyxia) in addition to the reactivity (sensitivity) of the receptor.

NaCN and ACh were examined for their ability to influence chemoreceptor activity at different temperatures. In interpreting data from drug experiments, the problem arose of whether to use the total discharge, as advocated by Paintal (19), or the averaged discharge evoked during stimulation. We measured both parameters and our data suggest that they follow the same qualitative pattern, within the dose range and temperatures studied, although there may be differences between the two at particular temperatures and/or doses.

ACh and NaCN were less effective in evoking a chemoreceptor response at lower temperatures, this being especially noticeable at 24°C. To explain the reduced response, one might speculate that the sensitivity of the receptor mechanism had been affected by the reduced temperature. Thus, if the sensitivity of the sensory mechanism is a function of spontaneous chemoreceptor activity, then at low temperatures metabolic activity, and hence sensory receptor sensitivity, would be reduced.

Whatever the explanation for the reduction in sensitivity to acetycholine, an important point is that even at 24°C, the lowest temperature investigated by Paintal (20), the stimulant is still active in evoking a response from chemoreceptor units. In contrast, Paintal concluded from his experiments that acetylcholine, because it elicited a very poor chemoreceptor response at low temperatures as compared with the effect evoked by hypoxia, is not likely to be a transmitter at the chemoreceptors. It should be noted that our experimental techniques differ from those of Paintal, who studied aortic chemoreceptors, used chloralose and sodium pentobarbital anesthesia, cooled the whole cat, and studied single doses of acetylcholine with the animal atropinized. We investigated carotid chemoreceptors, applied local temperature changes to the chemoreceptors, and obtained dose-response data with acetylcholine. It is of interest that Brown (4) has described how the cat superior cervical ganglion does not respond well at low temperatures (20°C) to injected ACh, although stimulation of the preganglionic nerve will elicit a contraction of the

nictitating membrane similar to that obtained at 37°C.

Krylov (16) found that the sensitivity of carotid chemoreceptors to ACh was not affected by reducing to 12°C the temperature of fluid perfusing a carotid bifurcation. Our results allow us to agree with the report to the extent that ACh does evoke responses at reduced temperatures, but the quantitative data show a definite reduction in the response elicited. Krylov also reported that at 20°C the chemoreceptor stimulant action of NaCN was absent. Our data at 24°C, in agreement with those of Nashat and Neil (18), obtained at 26°C, show the response evoked by NaCN is less at lower temperatures, but is nonetheless present. Perhaps at 20°C there is a sudden loss of response to NaCN, but not to ACh. The lowest temperature we investigated was 24°C, at which temperature the responses to ACh and NaCN are similarly reduced (Fig. 8).

The evidence from the temperature studies does not eliminate ACh from consideration as a chemoreceptor transmitter substance. Indeed, the observation that the μ values for spontaneous chemoreceptor activity, stimulation by hypercapnia and stimulation by ACh are so similar (Fig. 6), suggests that ACh and the physiological transmission process have the same rate-limiting step. This is compatible with endogenous acetycholine being a transmitter at chemoreceptors (6).

SUMMARY

The influence of local temperature changes on the activity of carotid chemoreceptors and baroreceptors has been investigated in anesthetized cats. Q_{10} values of 1.27 for the baroreceptors and 2.96 for the chemoreceptors were obtained and there was a linear relationship between chemoreceptor discharge and temperature when the data were plotted according to the Arrhenius equation.

At 24°C, asphyxia evoked a chemoreceptor discharge which, although it took longer, eventually reached the same maximal value as attained with asphyxia at 37°C. Both ACh and NaCN stimulated chemoreceptor activity at low temperatures, although the activity was less than that evoked at 37°C. The μ value (from Arrhenius equation) for ACh-induced chemoreceptor activity was very similar to that obtained with physiological stimulation of the chemoreceptors. The findings from this investigation are compatible with acetylcholine being a transmitter at chemoreceptors.

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EFFECTS OF SUBERYLDICHOLINE ON CAROTID BARORECEPTORS AND CHEMORECEPTORS

D. S. McQUEEN*

Department of Physiology, University of Utah College of Medicine, Salt Lake City, Utah 84132

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Summary—The actions of the nicotinic drug suberyldicholine (SDC) on carotid baroreceptors and chemoreceptors in the cat have been examined. Suberyldicholine has a direct stimulant effect on chemoreceptors, followed by a small delayed effect which is probably not due to SDC stimulating the superior cervical ganglion, since the effect is also present when the ganglio–glomerular nerves are cut. The direct stimulant effect on chemoreceptors was reduced by mecamylamine or tubocurarine, but not by moderate doses of atropine. Suberyldicholine thus affects chemoreceptor activity via actions at a nicotinic site.

There was no evidence of a direct action of SDC on carotid baroreceptors, even with high doses. Delayed increases in baroreceptor activity occurred with high doses of SDC, but these were secondary to the sizable changes in systemic blood pressure evoked by SDC.

The chemoreceptor-stimulating property of the nicotinic drug suberyldicholine (corconium or subecholine; bis-(2 tri-methylaminoethyl) suberate diiodide) has been described (ANICHKOV and BELLEN'KII, 1963; DARDYMOV and GER, 1964). However, there are reports suggesting that drugs which stimulate chemoreceptors when injected into the carotid bifurcation, also affect baroreceptors (DIAMOND, 1955; HEYMANS and NEIL, 1958).

McQUEEN (1970) used SDC to stimulate carotid chemoreceptors during a study of chemoreceptor reflexes and presented evidence which indicated it was unlikely that SDC, in the concentrations used to stimulate chemoreceptors, affected baroreceptors directly. Without electrophysiological evidence, however, the possibility could not be precluded. There was also a possibility that the drug was acting at the superior cervical ganglion to increase sympathetic nervous activity which would result in increased chemoreceptor activity (DALY, 1954).

The object of the present investigation was to use electrophysiological techniques to study the actions of SDC on carotid baroreceptors and chemoreceptors in the cat. The data obtained show that SDC stimulates the chemoreceptors directly; no direct action on the baroreceptors was observed in the dose range studied.

METHODS

Cats of either sex weighing between 1·9 and 4·6 kg were anaesthetized with sodium pentobarbitone (Diabutal, 40 mg/kg i.p.). A cannula was inserted into the trachea, low in the neck, and connected to a demand valve respirator (Ensco); spontaneous respiration being abolished by gallamine triethiodide (Flaxedil, 2 mg/kg i.v.). The animal was ventilated with

* Dorothy Temple Cross Research Fellow from Department of Pharmacology, University of Edinburgh, Scotland.

Address correspondence to: Department of Pharmacology, University of Edinburgh, George Square, Edinburgh.

air and the end-tidal CO_2 was continually monitored by an infra-red CO_2 analyzer (Beckman, LB-1), the P_{CO_2} being maintained at 20–25 mm Hg (atmospheric pressure 638–650 mm Hg) by adjusting the frequency and volume of respiration. A femoral artery was cannulated and connected to a pressure transducer, a record of arterial blood pressure being obtained on a pen recorder (Grass Model 5) and on magnetic tape (frequency response = d.c. to 2500 Hz). A femoral vein was cannulated and used for drug administration. The rectal temperature of the animal was monitored and maintained at 37°C.

Sinus perfusion and chemoreceptor stimulation

A common carotid artery was cannulated both ways and blood was pumped from the animal at constant flow through siliconized rubber tubing by a roller pump (Cole Parmer, model 7565). Before the blood was returned to the carotid artery, about 2 cm below the carotid bifurcation, it passed through a 16-cm spiral of polyethylene tubing which was positioned in close contact with a thermoelectric module (Borg–Warner Thermoelectric, Illinois; No. 920). The temperature of the blood at the carotid bifurcation was measured by a thermistor probe (Yellow Springs Instrument Co., No. 520) inserted via the external carotid artery, and maintained at 37°C by adjusting the thermoelectric module.

Sinus perfusion pressure was monitored and maintained between 120 and 160 mm Hg which allowed a flow rate of 1–3 ml/min depending on the particular animal. With the exception of the lingual and external carotid arteries, the blood vessels of the sinus region were not ligated. All the tubing was siliconized and heparin (150 units/kg i.v.) was administered initially and every 4 hr thereafter.

Chemoreceptor stimulants were injected into the tubing before the perfusion pump, against the direction of flow. Injections (0·1 ml over 1–2 sec) were repeated every 6 min, the drugs being prepared in modified Locke's solution: (NaCl, 6·0 g; KCl, 0·42 g; $CaCl_2$, 0·24 g; Trizma base, 6·0 g; normal HCl, 39 ml; distilled water to 1 litre; pH 7·41 at 37°C). Doses referred to are those of the salts.

The following drugs were used: acetylcholine chloride (Sigma), nicotine tartrate (K and K Labs), sodium cyanide (Merck), suberyldicholine diiodide, mecamylamine HCl (Merck, Sharp and Dohme), atropine sulphate (Mallinckrodt), (+)-tubocurarine chloride (Mann).

Recording of carotid sinus nerve activity

The carotid nerve on the same side as the perfused sinus was dissected from surrounding tissues under a binocular microscope. Unless otherwise indicated the ganglio-glomerular nerves (sympathetic nerves to the carotid bifurcation from the superior cervical ganglion) were cut. Exposed tissues were covered with warm mineral oil. The carotid nerve was sectioned centrally and fine strands were dissected from the nerve trunk. Recordings of multiple or single baroreceptor or chemoreceptor units were obtained using bipolar platinum electrodes. Sensory nerve discharges were amplified, displayed on an oscilloscope and recorded on tape. The output of the tape channel containing the action potentials was fed to a pulse height discriminator and a counter-timer coupled with a digital-analog converter, the output from which was obtained on the pen recorder. In addition, the output from the pulse height discriminator was fed to an analog-digital converter and spike frequency was analyzed by a digital computer (PDP-9).

Chemoreceptor units were identified by their aperiodic pattern of discharge (EYZAGUIRRE and LEWIN, 1961; BISCOE and TAYLOR, 1963) and their increase in discharge when sodium cyanide (5 μg) was injected into the carotid perfusion system. Baroreceptors were

identified by their synchronous discharge with pressure oscillations generated by the perfusion pump. Stopping the pump abolished or markedly reduced the discharge frequency, and sodium cyanide had no effect on the activity of these units.

For both types of receptor the discharge frequency of either single or multiple units was computerized. Data were expressed as the averaged control discharge (counts/sec) and the discharge elicited during the period of drug action (defined as the time from onset of the response until return to control level) was expressed as the average (counts/sec), maximal (counts/sec) and total counts.

RESULTS

Baroreceptors

Recordings of baroreceptor activity from 10 filaments (single or multiple units) in six cats showed no evidence of direct baroreceptor stimulation following single intra-carotid injections of SDC in the dose range 0·5–200 μg. The usual response was a slight fall in baroreceptor discharge following the injection and a concurrent fall in perfusion pressure. The decrease was attributable to the injection vehicle since injection of 0·1 ml Locke's solution elicited the same response, the change in viscosity was probably responsible for the slight fall in perfusion pressure and baroreceptor discharge.

After delay of 20–40 sec from the time a high dose of SDC (50–100 μg) entered the carotid bifurcation, there was an increase in baroreceptor activity which was associated with increases in sinus perfusion pressure and systemic blood pressure (Fig. 1C). This delayed

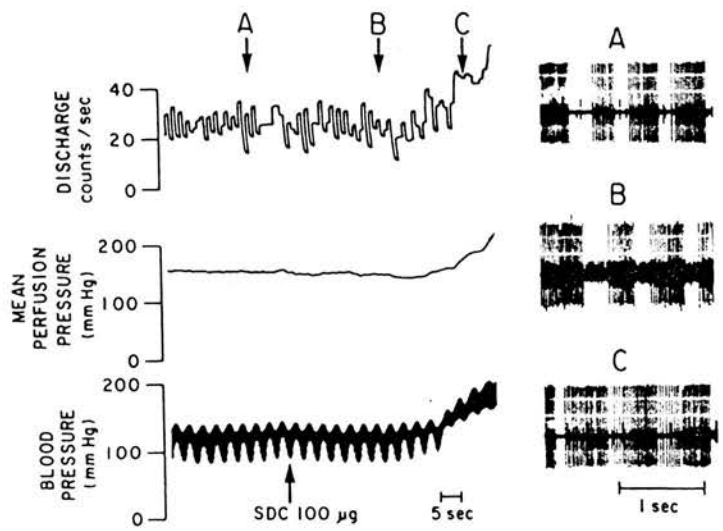

Fig. 1. Response of a single baroreceptor unit to an injection of 100 μg SDC into the carotid perfusion circuit. There was a 24-sec delay in the perfusion tubing before the drug reached the carotid bifurcation. Nerve discharge, mean sinus perfusion pressure and arterial blood pressure are shown. The panels to the right show recordings of nerve activity taken at the points indicated on the trace. Several small chemoreceptor units were present along with the large baroreceptor unit which had a triphasic action potential. A: during control period; B: during chemoreceptor stimulation by SDC, and it can be seen that the baroreceptor discharge frequency is not much affected although the chemoreceptors are strongly stimulated. When the perfusion pressure increases as a result of systemic actions of the drug, then baroreceptor activity is increased (C).

increase in baroreceptor activity was dose dependent and the discharge could be reduced or abolished by lowering the perfusion pressure (slowing the speed of the perfusion pump).

Chemoreceptors

Recordings of chemoreceptor activity from 16 filaments (single or multiple units) in 11 cats were obtained. Invariably SDC (0·2–200 μg) when injected into the carotid artery evoked a dose-dependent increase in chemoreceptor activity which was rapid in onset (Fig. 3A). The duration of the response varied from animal to animal and unit to unit. It depended on the perfusion flow rate, the rate of injection and the dose of stimulant; higher doses of SDC (20–200 μg) generally evoked responses with longer durations than those observed with lower doses. Analysis of the data from 79 tests in 11 cats showed than an average dose of 20 μg SDC (range 0·2–200 μg) evoked a response with an average duration of 13·8 sec (range 3·0–36·1 sec). The following aspects of the response were investigated.

Dose–response relationship. Qualitative examination of the data showed that SDC evoked a dose-dependent increase in chemoreceptor discharge. The problem arose concerning which parameter to select for quantitative analysis; that is, should one consider the averaged discharge, total discharge or maximal discharge? The data were examined using all three variables and it was found that a uniform pattern of response was obtained when the dose–response plots were compared. In some experiments the total count continued to increase with dose after the averaged discharge had reached a maximal value. Thus, in some instances, higher doses could evoke more activity but this was spread over a longer time such that the averaged discharge decreased.

Data obtained from single units were similar to those from multiple units in terms of shape of the dose–response curve and the dose which elicited maximal discharge—generally 50–100 μg SDC for most units. The type of response obtained is shown in Figure 2.

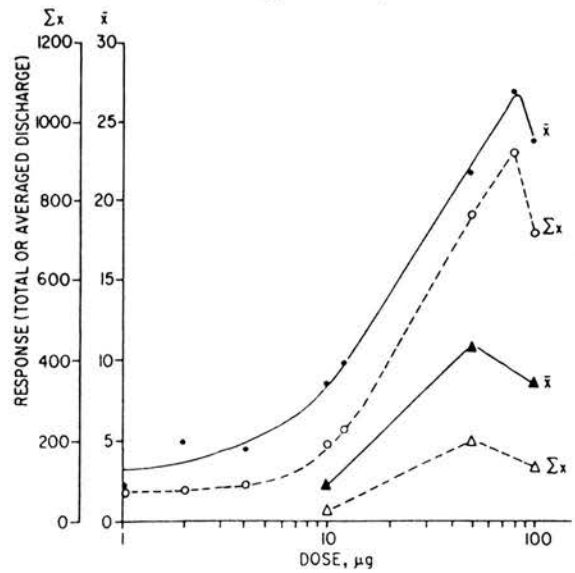

Fig. 2. Dose–response data from a recording of chemoreceptor activity (2 units). The averaged ($\bar{x}$, counts/sec) and total discharges ($\sum x$) in response to various doses of SDC were obtained before (○, ●) and after (△, ▲) mecamylamine (1 mg i.a.); the response duration averaged 23 sec (range 5·8–35 sec) before mecamylamine and 16 sec (range 12–19 sec) after mecamylamine. Both variables have similar dose–response curves.

The maximal value was not shown to avoid confusing the figure, but the curve for this parameter was similar to those shown and had a maximal value of 59 counts/sec at 50 μg SDC.

Blocking drugs. Small doses of some blocking drugs which have been reported to influence the response of chemoreceptors to stimulants were investigated. Mecamylamine (1 mg), when injected into the carotid perfusion system, reduced the response evoked by SDC (Fig. 2). In some instances this dose entirely abolished the response. Tubocurarine (1–2 mg i.a.) was also capable of markedly reducing the response of the chemoreceptors to SDC. Atropine, however, did not reduce the response until doses of 5–10 mg were injected into the carotid perfusion circuit, and even then there was only a short-lasting reduction in the response to SDC. Atropine (1 mg i.v.) appeared to potentiate the response.

Influence of ganglio–glomerular nerves. The majority of experiments in this investigation were performed with the sympathetic nerves to the carotid bifurcation (ganglio–glomerular nerves) cut because one of the objectives was to determine whether SDC could activate chemoreceptors directly. Two experiments were performed in which the nerves were left intact; results obtained were very similar to those from experiments in which the nerves were cut. The discharges elicited by SDC were similar in time-course and magnitude and a secondary response was sometimes obtained in both situations (Fig. 3). This secondary effect was associated with high doses of SDC and was not very obvious with lower doses nor with acetylcholine or sodium cyanide. It commenced about 20 sec after onset of the primary response and lasted longer. In most instances the primary response had terminated before onset of the secondary discharge and only the primary response was computerized.

Other effects

Suberyldicholine has been advocated as a chemoreceptor stimulant partly because of lack of tachyphylaxis. Repeated doses, it is claimed, evoke consistent responses while nico-

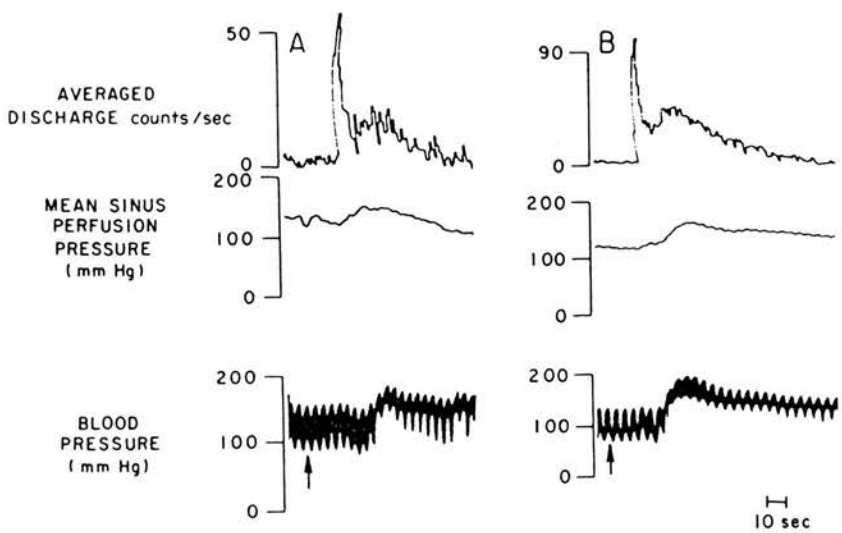

Fig. 3. Chemoreceptor activity evoked by SDC (50 μg) injected into the carotid perfusion circuit at the arrow in two different experiments. A: ganglio–glomerular nerves cut. B: ganglio–glomerular nerves intact. The secondary responses, present in both A and B, are among the largest observed.

tine becomes blocked on repeated administration. The ability to evoke consistent responses with SDC is attributed to destruction of the drug by cholinesterase (ANICHKOV and BELEN'KII, 1963; DARDYMOV and GER, 1964). In the present study it was found that doses of SDC could indeed be repeated frequently (20 μg once every minute) without tachyphylaxis. Nicotine, in doses evoking similar responses, generally but not invariably blocked the response on repeated administration.

Suberyldicholine when injected into the carotid perfusion circuit eventually reached the systemic circulation where it caused sizable increases in blood pressure (Figs. 1 and 3). Nicotine, in doses which evoked similar discharges, did not produce much alteration in blood pressure. The extensive cardiovascular changes caused by SDC would be disadvantageous during experimental studies requiring constant blood pressure.

DISCUSSION

Results from the present electrophysiological investigation confirm the observations of ANICHKOV and BELEN'KII (1963) and DARDYMOV and GER (1964) that SDC is a potent chemoreceptor stimulant. The possibility that the drug acts via the superior cervical ganglion to increase chemoreceptor activity (DALY, 1954) was precluded by cutting the sympathetic nerve supply to the carotid body. There was some delayed increase in chemoreceptor discharge, seen with higher doses of SDC, which did not appear to be entirely secondary to ganglion stimulation because it was also obtained when the ganglion–glomerular nerves were cut. This secondary response was readily distinguished from the primary response because it was delayed in onset, of longer duration and generally much less intense. The secondary response may be due to catecholamines, released from the adrenal medulla by SDC, reaching the carotid body or to SDC acting within the carotid body to release endogenous catecholamines. However, explanations such as recirculation of SDC could be advanced; in most instances the primary response had terminated before onset of any secondary effect and the cause of the secondary discharge was not investigated.

It was found that both mecamylamine and tubocurarine are capable of reducing the chemoreceptor response to SDC, while atropine had little effect except in high doses. The findings with mecamylamine are compatible with the report of DARDYMOV and GER (1964) that hexamethonium suppresses respiratory stimulation evoked by SDC acting via the carotid chemoreceptors. NISHI and EYZAGUIRRE (1970) reported that 10 mg atropine injected into the carotid artery of the cat reduced the chemosensory response to acetylcholine without causing a local anaesthetic action. If we preclude a local anaesthetic action, the reduced sensitivity to SDC following high doses of atropine in the present experiments may have been due to a nicotinic-blocking action of atropine.

ANICHKOV and BELEN'KII (1963) have suggested that the sensory receptor in the carotid body is more like the nicotinic receptor in ganglia than that at the neuromuscular junction. The present findings support the conclusion that SDC acts at a nicotinic site within the carotid body, but further quantitative pharmacology will be required to characterize the receptor.

There was no evidence that SDC affected the baroreceptors directly, even when large doses which evoked a maximal chemoreceptor response were injected into the carotid bifurcation. This supports the observation of MCQUEEN (1970), that in doses used to stimulate chemoreceptors, the baroreceptors are not affected directly by SDC. The delayed increase in baroreceptor activity seen with high doses of SDC seemed to be attributable to

the increase in sinus perfusion pressure. The increase in perfusion pressure might be due to retrograde flow from collaterals and/or constriction of vessels leaving the sinus. It might be argued that cutting the ganglio-glomerular nerves affected the ability of SDC to influence baroreceptor activity. This is not likely to be the case because the sympathetic nerves have very little or no influence on baroreceptor activity (FLOYD and NEIL, 1952).

ANICHKOV and BELEN'KII (1963) suggested that one of the advantages of SDC as compared with nicotine is its ability to evoke consistent chemoreceptor responses even when injected at frequent intervals. In the present investigation the ability of SDC to evoke consistent responses when injected at frequent intervals was confirmed, but SDC causes sizable cardiovascular changes which may be undesirable in certain situations. Suberyldicholine, however, does not have much action in the central nervous system and in clinical use as a respiratory stimulant (ANICHKOV and BELEN'KII, 1963) the hypertensive effect may be advantageous.

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A QUANTITATIVE STUDY OF THE EFFECTS OF CHOLINERGIC DRUGS ON CAROTID CHEMORECEPTORS IN THE CAT

By D. S. McQUEEN

From the Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

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SUMMARY

1. Conflicting qualitative evidence exists concerning the effects on chemoreceptor activity of some drugs which influence the cholinergic system. Quantitative evidence has been obtained in the present study which should resolve the conflict.

2. Experiments were performed in pentobarbitone-anaesthetized cats in which the activity of chemoreceptor units in the sinus nerve was used to assess chemoreceptor reponses. The effects of drugs on responses to I.A. ACh and NaCN were determined from dose–response data obtained from several animals and expressed as mean dose ratios.

3. The chemoreceptor response to ACh was slightly inhibited by atropine, α- and β-bungarotoxin and HC-3, almost completely suppressed by mecamylamine, and markedly potentiated by physostigmine.

4. Concomitant responses to NaCN were unaffected by atropine, β-bungarotoxin, mecamylamine or physostigmine. There was a slight inhibition following α-bungarotoxin and a potentiation after HC-3.

5. The results do not support the theory that ACh is an excitatory sensory transmitter in the carotid body.

INTRODUCTION

It has long been known that acetylcholine (ACh) stimulates the carotid chemoreceptors (e.g. Cordier & Heymans, 1935; Heymans, Bouckaert, Farber & Hsu, 1936; Anichkov & Belen'kii, 1963), and in 1938 Schweitzer & Wright made the suggestion that drugs or changes in the blood which stimulate the chemoreceptor nerve endings might produce their effects by liberating ACh as the chemical intermediary. This possibility has since been explored by many workers, but the evidence which has accumulated concerning the role of ACh in chemoreceptor sensory transmission is equivocal and controversial (see reviews by Douglas, 1954; Heymans,

1955; Heymans & Neil, 1958; Anichkov & Belen'kii, 1963; Eyzaguirre & Zapata, 1968*a*; Torrance, 1968; Biscoe, 1971; Howe & Neil, 1972).

Much of the controversy has centred around the conflicting results obtained from experiments in which drugs were used to modify the response of the chemoreceptors to physiological and pharmacological stimuli. Heymans & Neil (1958) expressed the opinion 'that ACh has nothing whatever to do with the normal transmission of the chemoreceptor impulses' and went on to add: 'The reasons for the conflict of evidence concerning the effects of anticholinesterases and ganglioplegic drugs on the activity and sensitivity of the glomus nerve endings to normal or pharmacological stimuli require further clarification.'

From consideration of the literature it seemed probable that the conflict resulted from conclusions being based on qualitative pharmacological data, obtained from a variety of preparations, which being difficult to evaluate have been liable to subjective interpretation according to whether the evidence was intended to support or reject involvement of ACh in chemosensory transmission. The purpose of the present study was to try to resolve this conflict by using a preparation that provides a reliable indicator of chemosensory activity to determine objectively the effects of cholinergic drugs on the chemoreceptors.

METHODS

Experiments were performed on cats of either sex weighing between 2·0 and 4·9 kg (mean 2·9 kg).

Anaesthesia. The animals were anaesthetized with pentobarbitone sodium, 42 mg/kg I.P., supplemented approximately every 1·5–2 hr during the experiment by 10% of the initial dose administered I.V.

General. A cannula was inserted into the trachea low in the neck and both femoral arteries were cannulated, one catheter being connected to a B.P. transducer (Bell and Howell, 4–442) and the other used for withdrawing blood samples for gas analysis. The signal from the transducer was displayed on a pen-recorder (Devices, M4) and recorded on one channel of an FM tape recorder (Tandberg, 100; frequency response d.c. to 1250 Hz).

A femoral vein was cannulated and used for drug administration. The lingual artery on the same side as the sinus nerve from which recordings were obtained was cannulated with a catheter (o.d. 0·75 mm) with the tip positioned in the common carotid artery 2 cm caudal to the carotid bifurcation and this position was confirmed post mortem. In some experiments another catheter was similarly positioned in the contralateral common carotid artery.

Respiration. The lungs were artificially ventilated with room air by a respiratory pump (S.R.I.) operating at 18 or 25 (later experiments) rev/min. End-tidal CO_2 was continually monitored by an infra-red CO_2 analyser (med 1A; Grubb Parsons) and maintained at 5% by appropriate adjustment of the pump stroke volume. Anoxic stimulation was achieved by replacing air with 100% N_2 as the ventilating gas and allowing the animal to inhale this for 2 min before returning to air.

Blood from a femoral artery was withdrawn approximately every hour during the

experiment and P_{a,CO_2}, P_{a,O_2} and pH estimated using a Radiometer gas monitor (BMS3 with PHM71 meter). Plasma bicarbonate was maintained between 20 and 25 mM either by adjusting the stroke of the respiratory pump or by the I.V. injection of molar sodium bicarbonate solution, the base deficit being calculated from the nomogram of Singer & Hastings (1948). The bladder was drained at regular intervals and rectal temperature maintained at $38 \pm 0{\cdot}5$ °C.

Recording of sinus nerve activity. The sinus nerve was identified and sectioned central to its junction with the glossopharyngeal nerve. Exposed tissues were covered with warm (37 °C) mineral oil. Electrical activity from single or multiple chemoreceptor units was recorded from filaments of the peripheral nerve using bipolar platinum-iridium electrodes. Sensory nerve discharges were amplified by an a.c. amplifier (Neurolog, Digitimer), displayed on an oscilloscope (Tektronix, 5103N) and recorded on one channel of the tape recorder.

Chemoreceptor units were identified by their random discharge (Eyzaguirre & Lewin, 1961; Biscoe & Taylor, 1963) and their increase in discharge frequency following injection of 5 μg NaCN into the ipsilateral common carotid artery. The ganglioglomerular nerves (one to three sympathetic nerves from the superior cervical ganglion to the carotid sinus region (Eyzaguirre & Lewin, 1961)) were cut as were nerves which were found to course between the carotid sinus and the nodose ganglion in some animals. The ganglioglomerular nerves were cut in order to eliminate reflex effects of sympathetic activity on carotid nerve discharge (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961). Results obtained from this preparation (Fig. 1) agree with reports that chemoreceptor discharge is largely independent of blood pressure, at least over the physiological range (Hornbein, Griffo & Roos, 1961; Biscoe, Purves & Sampson, 1970; Acker, Keller, Lübbers, Bingmann, Schulze & Caspers, 1973).

Single units were identified from the constant shape and amplitude of the action potential. In most of the multi-unit recordings it was found that a high dose of stimulant evoked a maximum discharge ($X_{max.}$ c.p.s.) which remained fairly constant during the course of an experiment. If a unit was recruited, or another ceased responding, $X_{max.}$ changed and this provided a quick method for monitoring the units and was used in conjunction with visual checking of the action potentials on the oscilloscope. Analysis was performed only if the number of units being examined, usually 2–3, remained constant throughout the experiment.

Data analysis. The output of the tape channel containing the action potentials was fed to a pulse height discriminator, the upper and lower levels at which the discriminator operated being indicated by Z axis modulation. The analogue output, which had been stored for 1 sec, was fed to a digital voltmeter (Schlumberger, A210) coupled to a data transfer unit (Schlumberger, 3240) which drove an Addo 5 punch.

Average discharge ($\bar{x}$) in the pre-stimulus or 'control' period, generally 20 sec, was computed (PDP-8 computer, Digital Equipment Corporation) from the punched tape. The average and total counts (Σx) were calculated for each response after its duration (t sec) had been determined from a histogram of the response, displayed by the computer on an x–y plotter (Complot, Houston Instruments). Responses were expressed as increments above the control level by subtracting the appropriate values, i.e.:

$$\Delta\bar{x} = \bar{x}\ (\text{response}) - \bar{x}\ (\text{control}),$$

$$\Delta\Sigma x = \Sigma x\ (\text{response}) - \Sigma x\ (\text{control}),$$

where Σx (control) $= \bar{x}$ (control) $\times t$ (response duration, sec).

A 'response' was defined as being from the first substantial (i.e. 3 times or more) increase above the mean control discharge frequency until the discharge returned to the pre-injection level. Beidler (1954) expressed chemosensory receptor activity

in terms of the integrated neural response (Σx) and the same approach has been advocated for arterial chemoreceptor studies by Paintal (1971) because Σx allows for differences in response duration. Data were therefore expressed in terms of $\Delta\Sigma x$, and also $\Delta\bar{x}$ because previous workers have generally used the averaged discharge when evaluating drug effects. Expressing the drug results in terms of both variables made it possible to determine whether there was an appreciable difference in the information they provided (see also McQueen, 1974).

Dose–response data. $\Delta\bar{x}$ and $\Delta\Sigma x$ were plotted against the $\log_{10}$ dose of the stimulant. A straight line was fitted to the points in the linear portion of the dose–response curve using the method of least squares. The slope (m) and intercept (c) were calculated for each line and from the equation for a straight line, $y = mx + c$, it was possible to calculate the response (y) elicited by a dose of stimulant (x).

A response in the central region of the control or pre-drug dose–response line was selected arbitrarily and the dose of stimulant required to match this response following drug administration was calculated from the post-drug dose–response line. The ratio of the dose required after drug administration to that required in the control state is the *dose ratio* and this provided an objective assessment of a drug's influence on responses evoked by chemoreceptor stimulants. Dose ratios obtained from different experiments were pooled and data presented as the mean ratio ± S.E. of mean. The S.E. of mean is given to provide an estimate of the scatter of individual ratios about the mean value, although they are not necessarily normally distributed. Data for each drug were obtained from several animals in order to provide an estimate of the population response.

Gallamine. The animals were paralysed during the experiment with gallamine (3 mg/kg I.V.), the dose being repeated as required, usually every 1–1·5 hr. This neuromuscular blocking drug was given to prevent muscle contractions, either spontaneous or caused by the close-arterial injections of ACh, from moving the nerve on the recording electrodes, and also to suppress spontaneous respiratory movements which are associated with fluctuations in end-tidal CO_2 and blood pressure.

Chemoreceptor discharge frequency obtained with the animal artificially ventilated and paralysed (end-tidal CO_2 5%, $P_{a.CO_2}$ about 34 mmHg) was very similar to that observed when it was breathing spontaneously, and remained relatively constant throughout the experiment – providing that the sympathetic nerve supply to the carotid body was cut and that mean blood pressure did not fall below 50 mmHg (see Fig. 3). Further, it was established that this dose of gallamine did not appreciably affect the response of the chemoreceptors to either ACh or NaCN (see Fig. 5).

Chemoreceptor stimulation. The effect of a chemoreceptor stimulant was determined by injecting 0·1 ml. of the solution into the common carotid artery via the lingual catheter and washing it in with 0·2 ml. Locke solution. Injections were made over 2 sec commencing at the peak of the inspiratory phase of the respiratory cycle and were repeated every 5 min. Other drugs were injected I.V. or intra-carotid over 5–20 sec and 10 min allowed before retesting the stimulants. The dead-space in the catheter was 0·1 ml. and it was flushed with 0·2 ml. Locke solution between injections.

Drugs. Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42 g; $CaCl_2$ 0·24 g; Tris base 6·0 g; N-HCl 39 ml.; distilled water to 1 l.; pH 7·41 at 37 °C). Doses referred to are those of the salts.

The drugs used in this investigation were: pentobarbitone sodium (Abbott Laboratories), gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide, atropine sulphate, physostigmine salicylate, carbamylcholine chloride (carbachol) (all B.D.H.); hemicholinium-3 (Aldrich), mecamylamine HCl (M.S.D.), α-bungarotoxin and β-bungarotoxin (Miami Serpentarium Laboratories, Miami, Florida, U.S.A.).

RESULTS

Experiments were performed on forty-three cats from which a total of sixty-nine recordings (seventeen single and fifty-two multiple units) of chemoreceptor activity were obtained.

Chemoreceptor responses to ACh and NaCN

In *all* the recordings ACh and NaCN were effective chemoreceptor stimulants. The threshold dose for stimulation varied slightly from recording to recording, generally being about 0·5 μg NaCN or 5 μg ACh. Maximum responses were evoked by 25–50 μg NaCN or 125–250 μg ACh, again with variation from one recording to another. NaCN elicited responses which had a longer latency to onset and lasted longer than comparable responses ($\Delta\bar{x}$) to ACh.

Vascular effects

The experiment shown in Fig. 1 illustrates that chemoreceptor activity in this preparation was largely independent of blood pressure. A large dose of ACh injected into the contralateral carotid artery caused a fall in blood pressure of the same magnitude as that seen following ipsilateral administration of the same dose, but without the chemoreceptor stimulation.

Since the chemoreceptor stimulants were not confined to the sinus region it was necessary to determine what effects they might have on recirculation. Experiments were performed on eight cats in which maximal or near-maximal doses of stimulant were injected into the contralateral carotid artery and the effect on chemoreceptor activity in the ipsilateral sinus nerve examined. It was found that these doses, which were higher than those used in dose–response studies, had little or no effect on the ipsilateral carotid body chemoreceptors, as can be seen from Fig. 1.

Analysis of chemoreceptor responses

It had been established by previous work (Diamond, 1955; McQueen, 1974) that there is a linear relationship between the $\log_{10}$ dose of stimulant and the chemoreceptor response over part of the dose–response curve. This was confirmed during preliminary experiments in the present investigation. However, the responses were rather scattered and fitting a dose–response line to the data by eye was subjective and, therefore, unsatisfactory. The relationship seemed to be sigmoidal, but the error involved in making the standard pharmacological assumption that response is linearly related to log dose over the central portion of the curve is

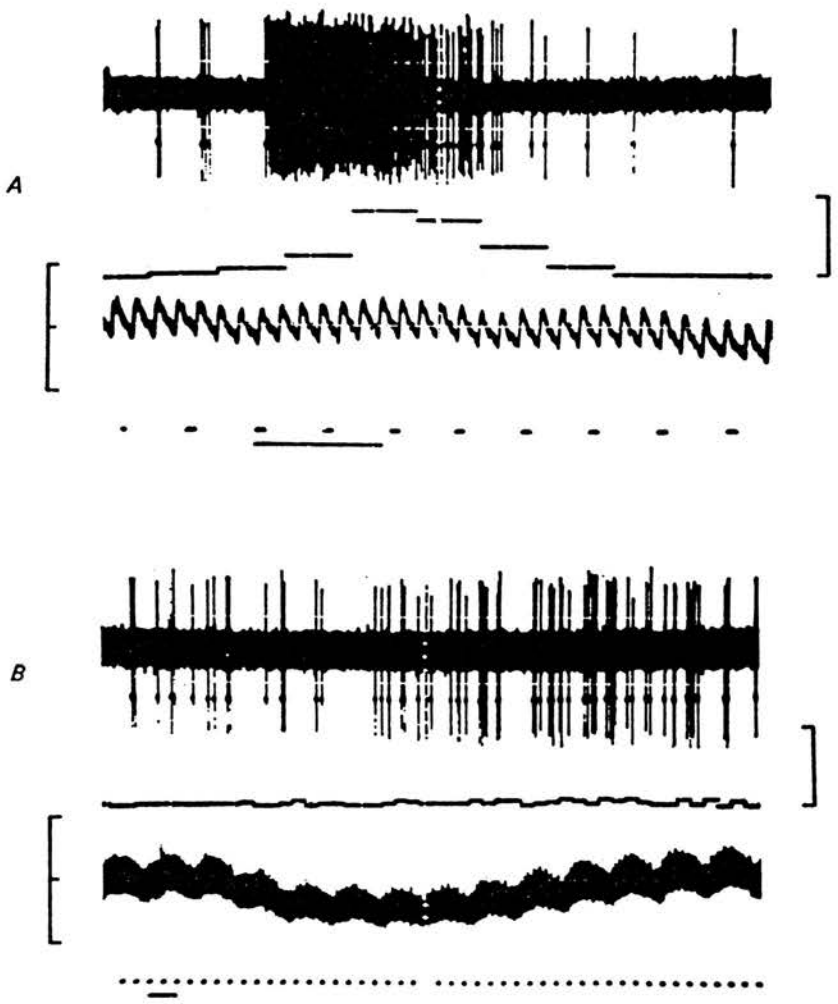

Fig. 1. Chemoreceptor unit from an experiment in which the influence of a high dose of stimulant on chemoreceptor activity in the contralateral sinus nerve was examined. *A*, ipsilateral injection of 125 μg ACh. *B*, the same dose administered on the contralateral side. Average spontaneous discharge was 0·8 c.p.s. before and 1·6 c.p.s. during the 20–30 sec period after injection of ACh. It can also be seen that hypotension did not appreciably affect chemoreceptor activity. Panels show from above downwards: nerve action potentials, the lower level at which the pulse height discriminator was operating being indicated by the brightening pulses; counter output in counts/sec, calibration on right of panel: 50 c.p.s.; B.P., calibration on left of panel: 0–100-200 mmHg; 1 sec time marker; injection marker.

likely to be negligible. Further experiments showed that dose–response curves obtained from one or two units were qualitatively similar to those obtained from multi-unit recordings (see Fig. 2).

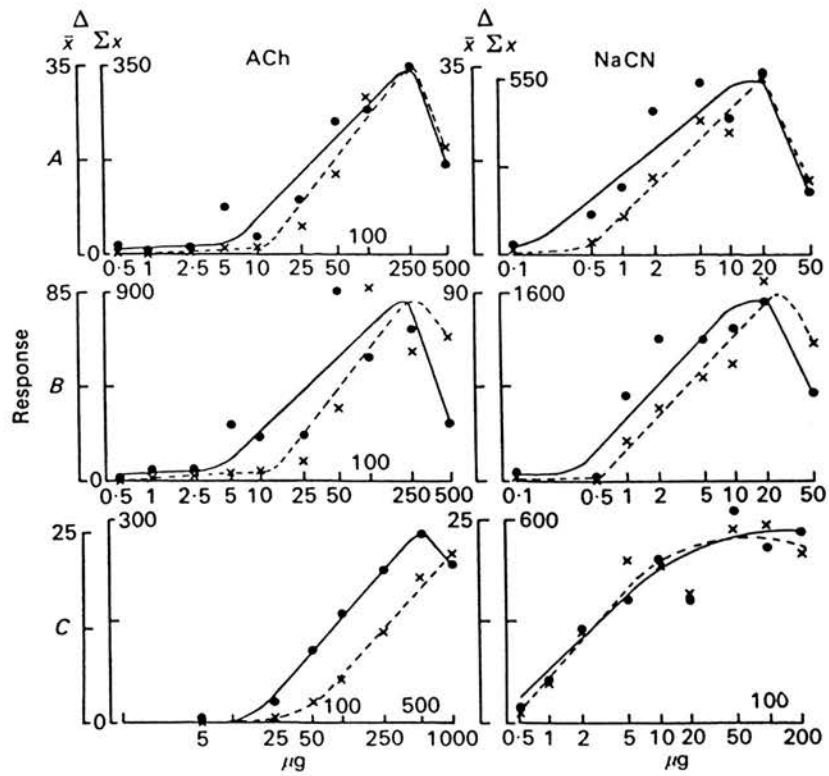

Fig. 2. Dose–response data obtained from two experiments. *A* and *B* are from the same recording, *A* being data from 2 units, *B* from 5 units. *C* is a single chemoreceptor unit obtained in a different experiment. Responses are plotted in this and subsequent figures ($\log_{10}$ scale) as $\Delta\bar{x}$ (●—●) and $\Delta\Sigma x$ (×- - -×) against the dose of either ACh or NaCN and the lines fitted to the points by eye.

To determine the constancy of the preparation's responsiveness during the long experiments required for dose–response studies, doses of stimulants were administered at regular intervals over several hours in three animals. Similar results were obtained, data from one of the experiments being shown in Fig. 3. These data provided an estimate of the variation in response to ACh and NaCN that might be expected during the long periods required for dose–response studies since other variables had been controlled as far as was possible and ACh, NaCN, gallamine and pentobarbitone had been administered, procedures which were to be common to

all other experiments. A variation of this magnitude in the dose–response ratio had to be accepted as inherent. The response to NaCN changed during the experiment, there being more counts elicited as the experiment progressed such that the $\Delta\Sigma x$ dose ratio decreased. However, the increased counts occurred over a longer time so that the $\Delta\bar{x}$ dose ratio was not affected.

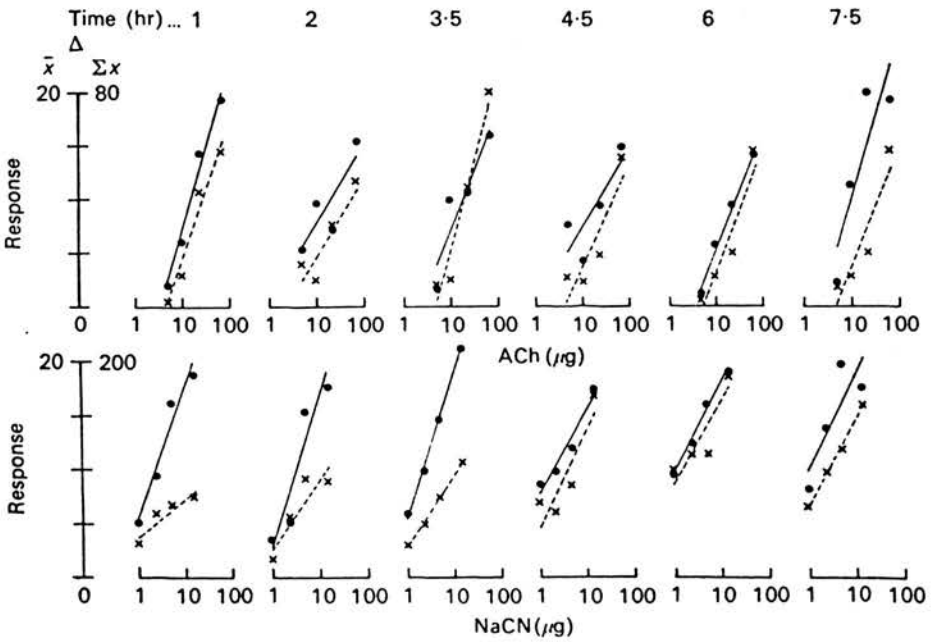

Fig. 3. Dose–response data from a single unit showing the effects of repeated doses of ACh and NaCN. Four doses of ACh and four of NaCN were injected in random order over a 45 min period, the sequence being performed six times during the course of a 9 hr recording. Gallamine was administered after each completed dose–response sequence - it was not present during the first cycle. Data are plotted as $\Delta\bar{x}$ (●—●) and $\Delta\Sigma x$ (×- - -×) against the dose of ACh or NaCN and the straight lines fitted by the method of least squares. The over-all mean spontaneous discharge, determined from the 20 sec control periods prior to injecting stimulants, was $2{\cdot}6 \pm 0{\cdot}2$ c.p.s., the average for individual sequences being in the range 1·8–3·4 c.p.s. The mean dose ratio (comparison against the first dose–response line) for ACh (20 μg) was $1{\cdot}4 \pm 0{\cdot}2$ ($\Delta\bar{x}$) and $1{\cdot}2 \pm 0{\cdot}1$ ($\Delta\Sigma x$). The corresponding ratios for NaCN (4 μg) were $1{\cdot}0 \pm 0{\cdot}1$ and $0{\cdot}6 \pm 0{\cdot}1$.

Drug effects

Drugs affecting the cholinergic system were used in this study and included antagonists such as mecamylamine, atropine and α-bungarotoxin, an anticholinesterase, and agents which interfere with the synthesis (HC-3) or release (β-bungarotoxin) of ACh. The results have been summarized in Fig. 5.

Mecamylamine. Mecamylamine itself caused inhibition of spontaneous activity lasting 10–15 sec which was followed by a slight increase in the discharge frequency, activity returning to control levels after about 30 sec. The lowest dose investigated was 0·1 mg/kg I.A. which gave dose ratios of 10 ($\Delta\bar{x}$) and 4·2 ($\Delta\Sigma x$) for ACh and 0·6 ($\Delta\bar{x}$) and 0·5 ($\Delta\Sigma x$) for NaCN, the reduction in response to ACh lasting for about 1·5 hr. In the remaining experiments mecamylamine was used at a dose of 1 mg/kg I.A. with, in some experiments, an additional 5 mg/kg I.A. administered later in the experiment.

Responses to ACh were blocked or substantially reduced by 1 mg/kg, it being impossible to determine the dose ratio since massive doses of ACh would have been required to overcome the inhibition and such doses (greater than 5 mg ACh I.A.) were not used since they would have caused substantial secondary effects. The ratio was therefore expressed as being > 10.

Whilst the response to ACh remained depressed for several hours, that to NaCN was not appreciably affected by mecamylamine (see Fig. 5). Even extremely high doses (up to 5 mg/kg I.A.), in excess of those reported by Nishi & Eyzaguirre (1971) as being capable of inhibiting the response to NaCN, had very little effect on the response to NaCN (see Fig. 4) even though tested over several hours.

A final attempt was made to inhibit the response to NaCN by injecting atropine (2 mg/kg) into the carotid artery about 1 hr after mecamylamine (5 mg/kg) had been given I.A. Nishi & Eyzaguirre (1971) described how in those chemoreceptor units in which the response to NaCN was not blocked by hexamethonium it was always depressed by injecting atropine (4 mg I.A., total dose). However, the results from the present experiments (see Figs. 4 and 5) showed only a slight inhibition of the cyanide effect, the response to ACh remaining inhibited by mecamylamine, and that to anoxic stimulation also being unaffected by the combination of mecamylamine and atropine.

The results show that mecamylamine, while reducing or abolishing the stimulation action of ACh on carotid chemoreceptor activity, had little or no effect on the response of these receptors to NaCN or anoxia.

Bungarotoxins. The response of chemoreceptors to ACh was reduced, although somewhat variably, by *α-bungarotoxin* (0·25 mg/kg I.A.). The reduction was not as great as that observed following mecamylamine (0·1 mg/kg I.A.). The response to NaCN was slightly inhibited, again with some variation from one experiment to another.

Responses to ACh and NaCN were examined before and after administering *β-bungarotoxin* (0·5 mg/kg). When the toxin had been present for 3 hr an additional dose of 0·5 mg/kg was injected I.A., and the animal was then subjected to anoxia for three 2 min periods over the course of

20 min. Nitrogen breathing evoked substantial and sustained increases in chemoreceptor activity. During the following 4 hr the anoxic stimulus was repeated from time to time, as were the chemical stimuli. The chemoreceptors were stimulated strongly over the course of several hours because at the neuromuscular junction it has been found that block of

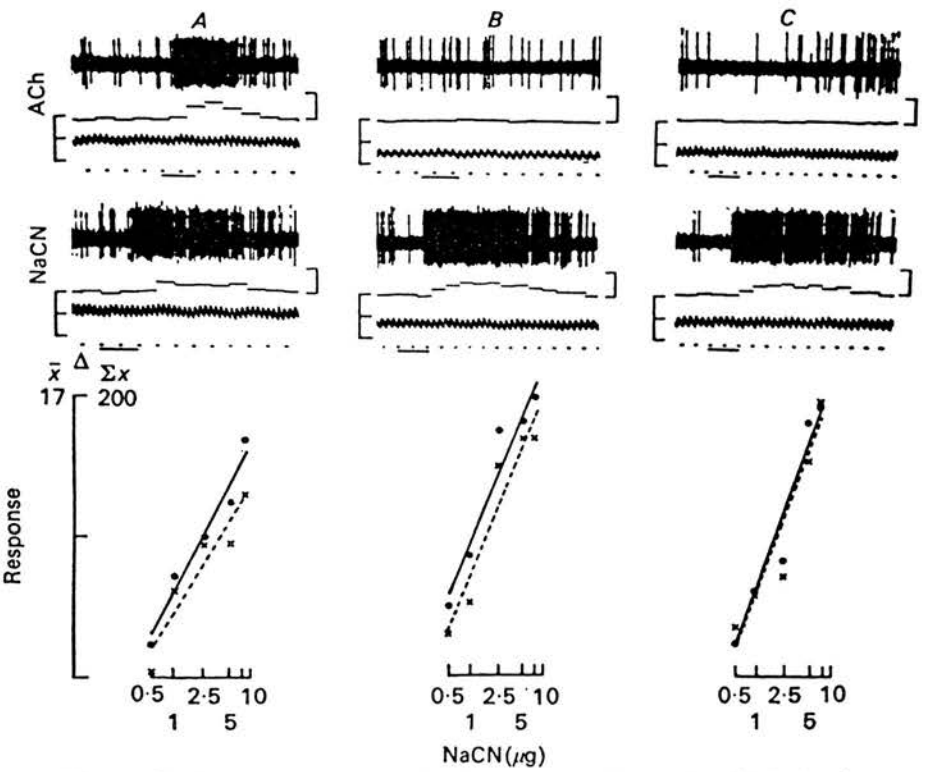

Fig. 4. Single chemoreceptor unit from an experiment in which the influence of high doses of mecamylamine and atropine on responses to ACh and NaCN was investigated. *A* shows the control response to ACh 250 μg and NaCN 5 μg, *B* the response to the same doses following mecamylamine (5 mg/kg I.A.) and *C* the response after the addition of atropine, 2 mg/kg I.A. Record details as for Fig. 1. The lower part of the figure shows the dose–response lines for NaCN, the details being the same as in Fig. 3.

transmission takes some time to occur and develops faster if the preparation is stimulated at a fairly high frequency (Chang, Chen & Lee, 1973).

The results obtained showed little or no change in the response of the receptors to either ACh or NaCN, and although there may be a slight inhibition of the ACh response, this was not intensified by the additional dose of β-bungarotoxin (see Fig. 5).

Hemicholinium-3 (HC-3). The effects of doses of 2 mg/kg I.V. (similar to that used by Eyzaguirre & Nishi, 1974) and 2 mg/kg I.A. were studied. Following administration of HC-3 the animals were made anoxic for three 2 min periods over the course of 20 min before testing the effects of ACh and NaCN. The responses to ACh were not obviously affected, while those to NaCN were found to be potentiated. Repeated anoxic stimuli did not alter either the response to anoxia or that to NaCN, even when continued for up to 5 hr after the I.A. dose of HC-3.

Additional data were obtained from four experiments in which a higher dose of 4 mg/kg was given I.A. In two of these animals α-bungarotoxin had previously been administered. Mean dose ratios of >10 ($\Delta\bar{x}$) and $1{\cdot}8 \pm 1{\cdot}1$ ($\Delta\Sigma x$) for ACh and $0{\cdot}1 \pm 0{\cdot}05$ ($\Delta\bar{x}$) and $0{\cdot}5 \pm 0{\cdot}1$($\Delta\Sigma x$) for NaCN were obtained. However, at this dose the blood pressure was very low and spontaneous chemoreceptor activity had increased such that it became difficult to compare responses before and after HC-3 and the data were not included in the results shown in Fig. 5. This inhibition of the ACh response may well have been due to a blocking action of high doses of HC-3 at the cholinergic receptor site (Martin & Orkand, 1961).

The results indicate that neither the response to NaCN or that to anoxia is depressed by HC-3, and indeed the response to NaCN is augmented.

Atropine. The effect of 1 mg/kg I.V. was investigated because it was intended to use this dose in experiments involving physostigmine – atropine would prevent excessive muscarinic actions, particularly hypotension, following ACh administration. Experiments were performed in which the 1 mg/kg I.V. dose was given and dose ratios determined for ACh and NaCN. Then an additional dose of 1 mg/kg was administered, this time close-arterial to the carotid body, and the dose ratios determined again. The results obtained (see Fig. 5) were rather variable, the overall pattern being a slight depression of the ACh response and no change or slight inhibition of the NaCN response.

Physostigmine (*Eserine*). The influence of physostigmine on the responses to ACh and NaCN was examined in atropinized cats (1 mg/kg I.V.). An initial dose of 0·2 mg/kg potentiated the response to ACh but not that to NaCN (see Fig. 5). An additional dose of 1 mg/kg I.A. further augmented the response to ACh, while that to NaCN remained more or less unaltered (see Fig. 5). The response to anoxia was not obviously affected by physostigmine, nor was the spontaneous discharge altered.

During three experiments the effect of carbachol, a cholinergic antagonist not destroyed by cholinesterase, was determined before and after physostigmine. The mean dose ratios obtained were $1{\cdot}0 \pm 0{\cdot}05$ ($\Delta\bar{x}$) and $1{\cdot}2 \pm 0{\cdot}2$ ($\Delta\Sigma x$) for the 0·2 mg, and $1{\cdot}7 \pm 0{\cdot}6$ ($\Delta\bar{x}$) and $1{\cdot}9 \pm 0{\cdot}4$ ($\Delta\Sigma x$) for

the 1 mg/kg dose of physostigmine. This implies that physostigmine potentiated ACh by inactivating cholinesterase and not by some non-specific action.

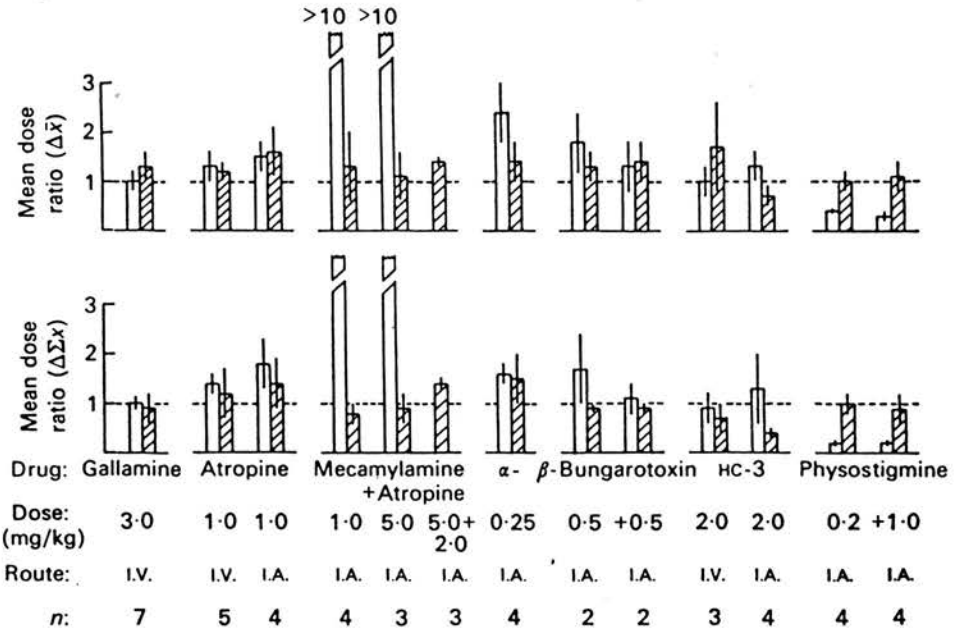

Fig. 5. Summary of the dose ratio data. The upper histogram gives the dose ratios ± S.E. of mean based on $\Delta\bar{x}$ and the lower histogram the ratios based on $\Delta\Sigma x$. Open rectangles are the ratios for ACh, the shaded rectangles the ratios for NaCN. The dashed line indicates the dose ratio of 1.

DISCUSSION

This study shows that drugs which affect the response of carotid chemoreceptors to ACh have little or no effect on the sensitivity of these receptors to NaCN or anoxia. The results are derived from a quantitative pharmacological investigation of drug effects on chemoreceptor activity and should be more reliable than those based on qualitative or semi-quantitative data. The extent to which this view is justified will be considered before discussing individual drug results.

Evaluation of drug effects

Electrical activity recorded from chemoreceptor units provides a more reliable indicator of chemosensory activity than do reflex changes, such as studied by Schweitzer & Wright (1938) and Byck (1961), because there are fewer secondary factors liable to influence the primary response and subtle changes can be detected. Single units were recorded in the present

investigation as were multiple units. There was no appreciable difference in the dose ratio data obtained by selectively counting single and multiple units (up to 6) in the same recording. This implies that the response of the population recorded is homogenous. Paintal (1967) found a difference between fast- and slow-conducting aortic chemoreceptor fibres in their responsiveness to ACh, the fast fibres being much less sensitive. The situation seems to be different in the sinus nerve where both types of fibre are sensitive to ACh and NaCN, the fast-conducting being slightly more sensitive (Sato, Fidone & Eyzaguirre, 1968; Fidone & Sato, 1969).

Chemoreceptor stimulants evoke variable responses and this presents problems when evaluating drug effects. If the variability resulted from secondary changes it should have been possible to minimize it by controlling these variables. However, even when secondary influences were controlled as far as was possible, some variation in response still occurred (see Fig. 3). The majority of authors who have performed drug studies on chemoreceptors support their conclusions by illustrating the response elicited by a particular dose of stimulant before and after administration of the drug being examined. The conclusions resulting from such single-dose studies may well be appropriate, but in view of the variability of chemoreceptor responses it would seem essential to provide quantitative data as supporting evidence rather than to show 'typical' responses. Dose ratios provide an objective estimate of a drug's effect on chemoreceptor activity.

Drug effects

Both ACh and NaCN were studied because they are classical stimulants around which much of the controversy concerning the ability of cholinergic drugs to influence chemoreceptor activity has revolved. Anoxia was also used and it provides a more physiological stimulus; the receptor's sensitivity to anoxia under the conditions in these experiments is akin to its sensitivity to NaCN. However, the response it evoked is gradual in onset with a long time course of action and is difficult to compare directly with the intense short-lasting responses evoked by ACh or NaCN.

Nicotinic antagonists

Probably the most controversial aspect of chemoreceptor pharmacology has been the conflicting evidence obtained from experiments involving ganglion blocking drugs. Some authors found that responses to ACh and NaCN (or hypoxia) were depressed by these agents (Landgren, Liljestrand & Zotterman, 1952; Joels & Neil, 1962; Nishi & Eyzaguirre, 1971; Eyzaguirre & Nishi, 1974) while others reported that although the response to ACh was depressed, that to NaCN or hypoxia was not (Moe, Capo &

Peralta, 1948; Douglas, 1952; Dontas & Nickerson, 1956; Anichkov & Belen'kii, 1963; Sampson, 1971). Tetraethylammonium (TEA) and hexamethonium were employed in the earlier studies while mecamylamine, which more readily penetrates membranes (Eyzaguirre & Zapata, 1968*b*), was used in the present experiments.

Mecamylamine completely inhibited the response to ACh without having much effect on the response to NaCN or anoxia even when high doses were used and several hours allowed for the antagonist to act (see Fig. 4). This finding is in agreement with the reports cited above that the chemoreceptor response to NaCN is not appreciably affected by ganglion blocking drugs. The argument of Moe *et al.* (1948) and Nishi & Eyzaguirre (1971) that the cyanide response is not depressed because the ganglion blocking drugs fails to reach an effective concentration at some 'intrinsic' site is not supported by the present results, and has previously been refuted by Gray & Diamond (1957).

There is a period of 1–2 min following the I.A. injection of high concentrations of drugs such as mecamylamine and atropine when the chemoreceptors do not respond to ACh, NaCN or anoxia. Nishi & Eyzaguirre (1970) also noted this and although they established it is not due to local anaesthesia, it seems unlikely that this short-lasting inhibition is a specific antagonism of endogenous ACh. It is probably a non-specific consequence of administering high concentrations of salts close-arterial to the carotid body.

The available evidence suggested that the action of *α-bungarotoxin* on cholinergic nerves is confined to the motor nerve of skeletal muscle (Chang & Lee, 1963). However, it was considered worth investigating the action of this substance on the chemoreceptor cholinergic receptor because D-tubocurarine is effective at this site and α-bungarotoxin appears to act at the same site as D-tubocurarine at the neuromuscular junction (Simpson, 1974). α-Bungarotoxin inhibited the response to ACh to about the same extent as did atropine. The inhibition was slight when compared with that caused by mecamylamine, and further studies are needed to establish whether the toxin is active at the same site as mecamylamine, or whether it acts elsewhere to reduce the response to ACh and, to a lesser extent, that to NaCN.

Muscarinic antagonist

Although it is generally agreed that the cholinergic receptor site in the carotid body is nicotinic (see Anichkov & Belen'kii, 1963), there are reports that *atropine* is effective in reducing chemoreceptor sensitivity to ACh and NaCN (Liljestrand, 1951; Landgren *et al.* 1952; Eyzaguirre & Nishi, 1974). This could be taken to imply that part of the response to ACh is mediated by muscarinic receptors, although it is more likely to be

a consequence of local anaesthesia (Heymans, Delaunois, Martini & Janssen, 1953) or antagonism at a nicotinic receptor site (Nishi & Eyzaguirre, 1971; McQueen, 1974). The results obtained showed atropine slightly inhibited the response to ACh, and to a lesser extent, that to NaCN. It is not known whether the inhibition observed was due to effects on nicotinic receptors or to some other action of atropine.

HC-3 and β-bungarotoxin

HC-3 has been used by Eyzaguirre and co-workers (Eyzaguirre & Zapata, 1968*b*; Nishi & Eyzaguirre, 1971; Eyzaguirre & Nishi, 1974) to prevent the synthesis to ACh in the carotid body. They found that treatment with HC-3 followed by periods of hypoxia (to deplete the stores of ACh) resulted in a diminished response to NaCN without affecting the response to ACh. In the present experiments the response to NaCN was depressed, sometimes markedly, as shown by $\Delta\bar{x}$ dose ratio data (Fig. 5). At first sight this agrees with Nishi & Eyzaguirre's findings. However, if the $\Delta\Sigma x$ ratio is considered, it can be seen that the response was slightly potentiated. The explanation for the difference between ratios is that the integrated response was greater but spread over a longer time, so that although the $\Delta\Sigma x$ ratio decreased, the $\Delta\bar{x}$ ratio increased. Intra-arterial HC-3 further augmented the response to NaCN, both variables giving similar dose ratios.

The potentiation of the NaCN response by HC-3, and also by low doses of mecamylamine, could mean that the cyanide response is normally partially suppressed by a cholinergic mechanism. This is, however, only speculation since the cause of the potentiation following administration of these drugs has not been determined.

β-Bungarotoxin acts at the neuromuscular junction presynaptically and prevents the release of ACh (Chang *et al.* 1973). If it acts in a similar way at the chemoreceptor it would be expected that any stimulant which acts by releasing endogenous ACh would be rendered ineffective. The response to exogenous ACh should not be affected since there is no evidence of post-synaptic receptor blockade (Simpson, 1974). In the doses used in the chemoreceptor experiments β-bungarotoxin did not affect the cyanide response. The negative result could mean that activity evoked by NaCN is not dependent on ACh, but this would be the case only if β-bungarotoxin at the dose level studied can be shown to be effective in preventing the release of ACh in the carotid body. Further studies are required.

Anticholinesterases

The possibility that ACh might be involved in physiological transmission at chemoreceptors was raised by Schweitzer & Wright (1938) because of

the similar respiratory effects evoked by neostigmine and ACh. Others found the response to hypoxia was potentiated by physostigmine (Liljestrand, 1951; Landgren *et al.* 1952) whereas Heymans, Bouckaert and Pannier (1944) concluded from experiments in dogs that although the response to ACh was potentiated, that to other stimulants was not. Present results demonstrate that the response to exogenous ACh is potentiated by *physostigmine* while that to NaCN is definitely not influenced. Both acetylcholinesterase and pseudocholinesterase are located in the cat carotid body (Biscoe & Silver, 1966).

In conclusion, the increased chemoreceptor activity evoked by NaCN was not affected by either atropine, mecamylamine or physostigmine in the doses used, but was augmented by HC-3. Responses to ACh were quite clearly inhibited by mecamylamine and potentiated by physostigmine, this being in accord with the consensus in the literature. The thesis of this study is that conflicting evidence concerning the actions of cholinergic drugs on the chemoreceptors arises from subjective interpretation of qualitative data. Since conclusions reached in the present work are based on objective evidence obtained from quantitative pharmacological data, they should help to resolve the conflict of evidence referred to by Heymans & Neil (1958).

Cholinergic theory of chemosensory transmission

It should be appreciated that it was not the intention to investigate the mechanism whereby ACh, NaCN, or anoxia excite chemosensory nerves. However, the results obtained make it extremely unlikely that endogenous ACh is involved as an intermediary in the chemoreceptor response to NaCN and, therefore, do not support the theory that ACh is an excitatory sensory transmitter in the carotid body. Some of the most persuasive evidence in favour of the cholinergic theory has come from pharmacological studies on isolated carotid bodies *in vitro* (Eyzaguirre & Zapata, 1968*b*), but unfortunately direct comparison of this with evidence from the present experiments is not feasible because of the completely different experimental conditions.

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The effect of α-flupenthixol on the response of carotid chemoreceptors to acetylcholine, sodium cyanide and dopamine in the cat

By R. J. Docherty and D. S. McQueen. *Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ*

Dopamine (DA) may modulate sensory activity of cat carotid chemoreceptors (Zapata, 1975; Osborne & Butler, 1975). If the theory advanced by Osborne and Butler is correct and sensory activity is indeed kept

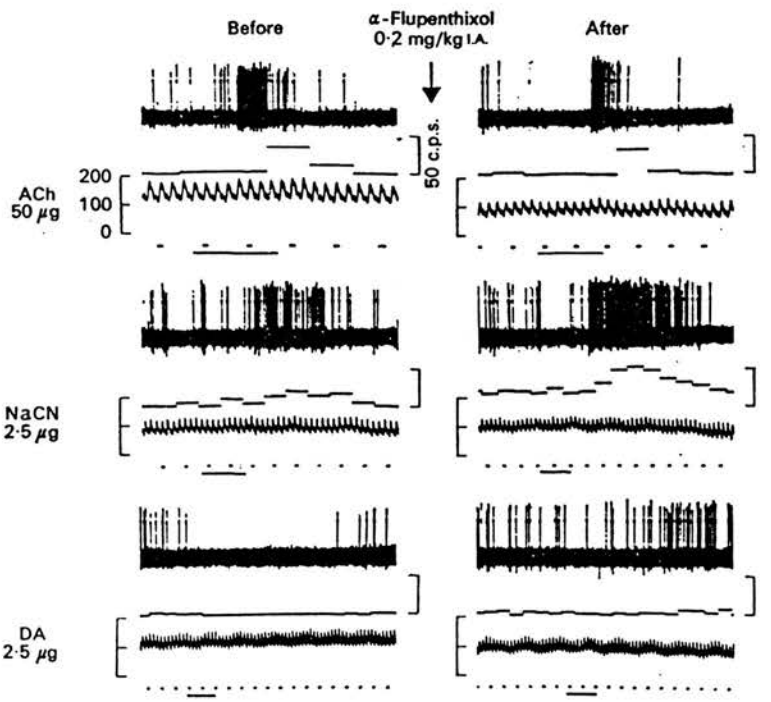

Fig. 1. Response of a chemoreceptor unit before (mean spontaneous discharge $2{\cdot}8 \pm 0{\cdot}1$ c.p.s.) and after ($2{\cdot}7 \pm 0{\cdot}1$ c.p.s.) α-flupenthixol. Panels show: action potentials; counter output; B.P.; 1 sec and injection markers.

suppressed by the continuous release of DA, then block of the DA receptor should substantially increase spontaneous chemoreceptor activity and also, according to the theory, markedly reduce the response to ACh. We used α-flupenthixol, a potent inhibitor of DA in the C.N.S. (Iversen, 1975) to block the inhibitory effect of DA.

Experiments were performed on eight pentobarbitone-anaesthetized cats in which ganglio-glomerular nerves were cut, the animals were

[P.T.O.

artificially ventilated and a gallamine (3 mg/kg) administered. Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve and stimulants injected into the ipsilateral carotid artery. The results showed that α-flupenthixol (0·2 mg/kg I.A.) abolished the inhibitory effect of DA while the response to NaCN was augmented and that to ACh slightly reduced. (Fig. 1).

Providing that exogenous DA is acting at the same site(s) as endogenous DA, the results suggest that while there may be some tonic inhibition of sensory activity by DA, this is not substantial. It is also unlikely that ACh or NaCN act to any appreciable extent by inhibiting DA release.

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Inhibitory effects of acetylcholine and dopamine on rabbit carotid chemoreceptors

By R. J. Docherty and D. S. McQueen. *Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ*

During experiments in pentobarbitone-anaesthetized rabbits we observed that intracarotid (I.C.) injection of acetylcholine (ACh, 5–250 μg) caused an immediate

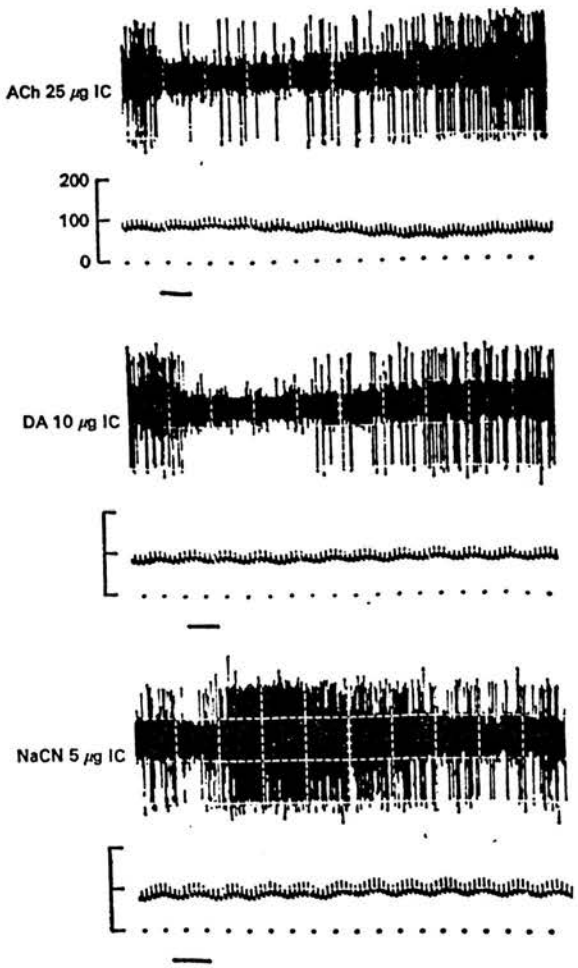

Fig. 1. Recording of chemoreceptor activity obtained from a rabbit, illustrating responses to ACh, DA and NaCN. Panels show: action potentials, B.P.; 1 sec and injection markers.

inhibition of respiration, whereas NaCN (1–25 μg I.C.) markedly stimulated respiration. Cutting the ipsilateral sinus nerve abolished the response to NaCN and greatly reduced the inhibitory action of ACh. The possibility that ACh was inhibiting chemosensory activity was investigated by recording from the peripheral end of a cut sinus nerve.

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ACh (5–250 μg I.C.) caused a dose-dependent inhibition of discharge (Fig. 1). With doses greater than 100 μg the inhibition was preceded by a slight transient increase in discharge. Atropine (1–5 mg/kg I.V.) slightly reduced the response to ACh. Dopamine (DA, 10 μg I.C.) also inhibited chemoreceptor activity. To determine whether the ACh-induced inhibition was secondary to DA release, we administered the DA antagonist α-flupenthixol (0·25–0·5 mg/kg I.V.). DA-induced inhibition was abolished, whereas that caused by ACh was only slightly reduced.

In contrast to other species where ACh increases chemosensory activity, and has been proposed as an excitatory transmitter (see review by Biscoe, 1971), our evidence shows that ACh has an inhibitory effect on rabbit chemoreceptors, implying that endogenous ACh is unlikely to be an *excitatory* transmitter in this species.

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INHIBITORY ACTION OF DOPAMINE ON CAT CAROTID CHEMORECEPTORS

BY R. J. DOCHERTY AND D. S. McQUEEN

From the Department of Pharmacology, University of Edinburgh, 1, George Square, Edinburgh EH8 9JZ

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SUMMARY

1. The influence of some drugs which affect the dopaminergic system was studied on chemosensory responses to dopamine (DA), acetylcholine (ACh), sodium cyanide (NaCN) and hypoxia during experiments on pentobarbitone anaesthetized cats in which chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve.

2. Spontaneous chemosensory activity was inhibited in a dose-dependent manner by DA (0·5–5 μg, I.A.). Higher doses (10–50 μg) caused a delayed increase in discharge and were associated with inconsistent inhibitory responses.

3. The DA antagonist α-flupenthixol (0·2 mg/kg, I.A.) blocked the inhibitory response to DA without affecting either the spontaneous discharge frequency or the response to ACh. The effect of NaCN was potentiated, and during hypoxia chemoreceptor activity increased more rapidly, although the maximum frequency attained was not appreciably different from control values. Similar results were obtained with haloperidol (0·5 and 1·0 mg/kg, I.V.).

4. Higher doses of α-flupenthixol (0·5–1·0 mg/kg, I.A.) increased spontaneous chemoreceptor activity, but this was regarded as a non-specific effect of the drug since at these doses the inhibitory effect of 5-hydroxytryptamine (5-HT) was also abolished.

5. The animals were exposed to alternate periods of hypoxia and hyperoxia following administration of the tyrosine hydroxylase inhibitor α-methyl *p*-tyrosine (AMPT, 0·2–10 mg/kg, I.A.). The inhibitory response previously evoked by amphetamine was abolished, and electron microscopic studies showed a great reduction in the number of dense-cored granules, both of which suggested that DA levels in the carotid body had been substantially reduced. Responses to NaCN and hypoxia were slightly potentiated following AMPT, but neither spontaneous activity nor the response to ACh was affected.

6. Apomorphine (0·05–0·2 mg/kg, I.A.) inhibited the chemoreceptor discharge for up to 45 min, an effect which was antagonized by α-flupenthixol (0·2 mg/kg, I.A.), implying it resulted from DA receptor stimulation. Although responses to NaCN, hypoxia and higher doses of ACh were reduced following administration of apomorphine, the reduction was not very marked.

7. These results are not compatible with the theory of Osborne & Butler (1975), that in normoxia DA is tonically released in the carotid body and suppresses spontaneous chemosensory activity.

8. It is concluded that DA modulates chemosensory activity by influencing the rate of increase in discharge, without affecting maximum discharge frequency. The mechanism whereby DA is released in response to increased chemosensory activity remains to be established.

INTRODUCTION

A new theory of carotid body chemoreceptor activity has recently been advanced (Butler & Osborne, 1975; Osborne & Butler, 1975). This proposes that in normoxic conditions dopamine (DA), which is known to be present in the cat carotid body (Chiocchio, Biscardi & Tramazzani, 1966; Zapata, Hess, Bliss & Eyzaguirre, 1969), is tonically released from Type 1 cells and suppresses the spontaneous discharge of afferent nerve endings; in hypoxic conditions DA release is attenuated and consequently afferent nerve activity increases.

The present pharmacological study was undertaken to test this theory by investigating the response of cat carotid chemoreceptors to acetylcholine (ACh), sodium cyanide (NaCN), DA, and hypoxia, before and after administering drugs which influence the dopaminergic system. A preliminary report on some of the results has been made to the Physiological Society (Docherty & McQueen, 1977).

METHODS

The experimental details have previously been described fully (McQueen, 1977) and only a brief summary follows. Experiments were performed on cats of either sex weighing between 2·0 and 4·4 kg (mean weight 2·9 kg) which were anaesthetized with sodium pentobarbitone (42 mg/kg, I.P.), supplemented approximately every 1·5–2 hr during the experiment by 10% of the initial dose administered I.V. Blood pressure was recorded from a femoral artery, the bladder drained at regular intervals and the rectal temperature maintained at $38 \pm 0{\cdot}5$ °C.

A sinus nerve was cut centrally and electrical activity from single or multiple chemoreceptor units was recorded from filaments of the peripheral nerve. The ganglioglomerular nerves were cut in order to eliminate the influence on chemoreceptor discharge of reflex changes in sympathetic nerve activity (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961). The animals were artificially ventilated either with air, 5% O_2 in 95% N_2 (hypoxic stimulus), or 100% O_2 (hyperoxia). They were paralysed by gallamine triethiodide (3 mg/kg I.V.).

Drug solutions (0.1 ml.) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded and washed in with 0·2 ml. modified Locke solution which had been bubbled with 5% CO_2 in air in a water-bath at 37 °C (solution P_{CO_2} 32 mmHg; P_{O_2} 132 mmHg; pH 7·0). Injections were made over a 2 sec period commencing at the peak of the inspiratory phase of the respiratory cycle.

Nerve activity was recorded on magnetic tape (d.c. to 1250 Hz) and subsequently analysed to provide data concerning the change in integrated discharge ($\Delta\Sigma x$) following drug administration. Responses were plotted against $\log_{10}$ dose and straight lines fitted by the method of least-squares to data obtained before and after the test procedures. A response in the central region of the control dose–response line was selected arbitrarily and the dose of stimulant required to match this response following drug administration was calculated from the post-drug dose–response line. The ratio of the dose required after drug administration to that required in the control state is the *dose ratio*. Dose ratios obtained from different experiments were pooled and data presented as the *mean dose ratio* ± S.E. of mean. These ratios provided a quantitative estimate of the effect of a drug on chemoreceptor responses to ACh and NaCN. Control values were determined by calculating the average discharge in the 20 sec period preceding the test stimulus. All the individual values were pooled to provide a mean control ± S.E. of the mean for spontaneous chemoreceptor activity which could be compared with that obtained following administration of one of the drugs being studied. Inhibitory responses were calculated by determining the integrated discharge (Σx) observed during the response period (t sec), defined

as the time from onset of inhibition until return to control average discharge ($\bar{x}$ c.p.s.). The inhibition was then expressed as:

$$-\Delta\Sigma x = \Sigma x - (\bar{x}.t).$$

Drugs. Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42 g; $CaCl_2$, 0·24 g; Tris base, 6·0 g; normal HCl 39 ml.; distilled water to 1 l.) excepting α-flupenthixol and α-methyl-*p*-tyrosine which were dissolved in 0·9 % aqueous sodium chloride, and haloperidol which was dissolved in 1 % aqueous tartaric acid.

The drugs used were: sodium pentobarbitone (Abbott Laboratories), gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide (B.D.H.), dopamine hydrochloride (Koch Light), α(cis)-flupenthixol dihydrochloride (Lundbeck & Co.), apomorphine hydrochloride (Macfarlan Smith), haloperidol (Janssen), 5-hydroxytryptamine creatinine sulphate, D-amphetamine sulphate (Sigma), DL-α-methyl-*p*-tyrosine (Labkemi A.B.).

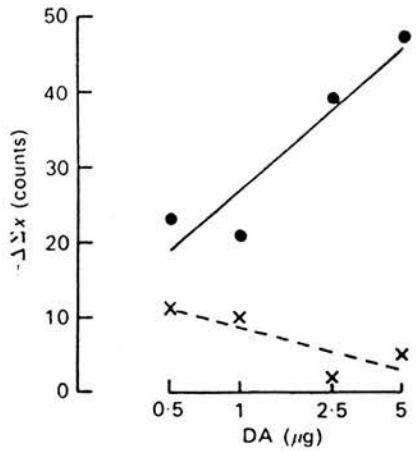


Fig. 1. Dose–response lines showing the inhibitory effect of low doses of DA (plotted on a $\log_{10}$ scale) on a single chemoreceptor unit before (●—●) and after (×---×) administering α-flupenthixol (0·2 mg/kg, I.A.). The average spontaneous discharge was 2·5 ± 0·3 c.p.s. before and 2·8 ± 0·3 c.p.s. after α-flupenthixol. Straight lines were fitted to the data by the method of least-squares.

RESULTS

Experiments were performed on fifteen cats from which a total of seventeen recordings (eight single, nine multiple units) of chemoreceptor activity were obtained.

Dopamine

DA reduced spontaneous discharge frequency in all fifteen experiments, the magnitude of the inhibition being dose-dependent over the range 0·5–5 μg I.A. (see Fig. 1) and the effect lasting for 5–45 sec. Higher doses (10–50 μg I.A.) caused a delayed or secondary increase in discharge which curtailed the primary inhibition and gave inconsistent responses. A secondary increase in chemoreceptor discharge was also observed by Zapata (1975) in his experiments with DA on the carotid body *in vitro*, so it was unlikely that this delayed effect was attributable to vascular changes. Zapata also reported that frequent administration of DA (dose unspecified) caused inhibitory responses to be converted to biphasic responses. In view of his findings and our observations with high doses of DA, we confined our studies to low doses of DA

administered infrequently and obtained consistent responses to both DA and the chemoreceptor stimulants.

Early in experiments ungassed Locke solution caused a very slight inhibition of spontaneous chemoreceptor activity, but later it caused an inhibition similar to that evoked by low doses of DA. It was therefore necessary to use Locke solution which

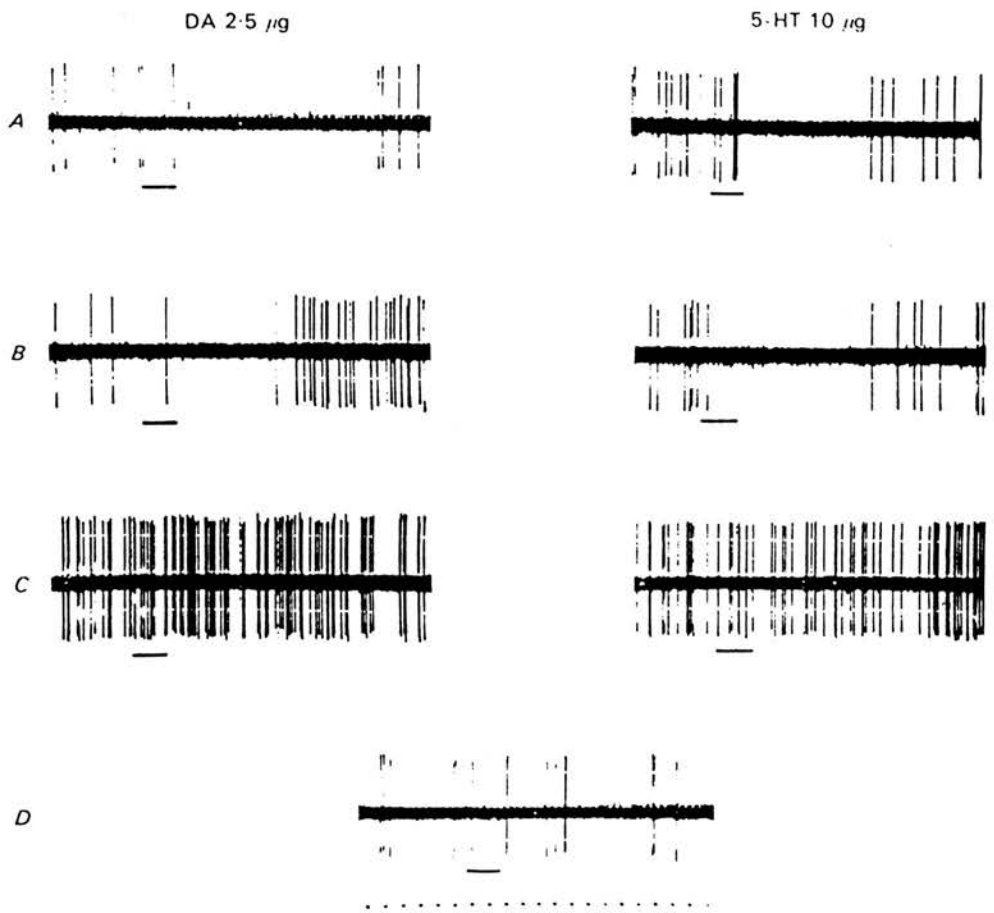
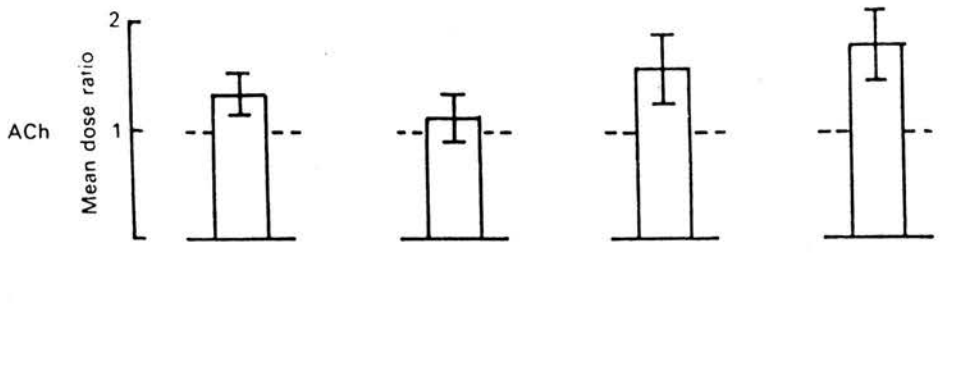


Fig. 2. Responses of a single chemoreceptor unit to DA and to 5-HT before and after α-flupenthixol. *A*, control, *B*, after 0·05 mg/kg α-flupenthixol, I.A. and *C*, after an additional 0·5 mg/kg. *D* shows the effect of 0·3 ml. Locke solution, I.A. Injection periods are represented by the horizontal bar below each panel and 1 sec timing marks are shown below *D*.

had been bubbled with 5% CO_2 in air to wash in drug solutions (the gassed solution did not cause any appreciable inhibition, even during long experiments; see Fig. 2). There was a slight increase in mean B.P. which commenced about 10 sec after an I.A. injection of high doses of DA (> 10 μg). With lower doses there was no obvious change in mean B.P.

α-Flupenthixol

α-Flupenthixol is a potent DA antagonist (Møller Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland & Christensen, 1973; Miller, Horn & Iversen, 1974) which acts post-synaptically (House & Ginsborg, 1976) and has very little anticholinergic activity (Iversen, 1975). We used it to reduce the inhibitory action of DA and, while responses to DA were suppressed, investigated the effects of ACh, NaCN and hypoxia. Dose ratios showing the influence of α-flupenthixol on the responses were calculated from the central region of $\Delta\Sigma x$ dose–response lines and the results obtained from ten experiments are summarized in Fig. 3. Responses to DA could not be expressed in the same way because after α-flupenthixol the slope of the DA dose–response line became negative, reflecting the tendency for DA to evoke excitatory responses under these conditions (see Figs. 1 and 2).



α-Flupenthixol (mg/kg I.A.):		0·05	0·2	0·5	1·0
Mean control discharge (c.p.s.) ± S.E of mean	Before:	3·7±1·3	4·7±0·8	3·0±0·6	6·8±1·5
	After:	4·3±1·0	5·9±1·1	3·4±0·6	10·3±1·3
Experiments (*n*)		=5	5	4	4

Fig. 3. Pooled dose ratio data showing the effect of various doses of α-flupenthixol on chemoreceptor responses to ACh and NaCN. Data are presented as mean dose ratios ± S.E. of the mean, and the dashed line represents a dose ratio of 1.

Low doses of α-flupenthixol (0·05 mg/kg, I.A.) reduced the inhibitory action of DA and showed up a small delayed increase in discharge (see Fig. 2), without appreciably affecting either spontaneous chemoreceptor activity or the responses to ACh and NaCN (Figs. 2 and 3). A dose of 0·2 mg/kg, I.A. abolished the inhibitory effect of DA (see Fig. 1), potentiated the response to NaCN, and was without much effect on the spontaneous discharge frequency or the response to ACh. Higher doses of antagonist (0·5 or 1·0 mg/kg, I.A., additional to 0·05 and 0·2 mg/kg respectively) resulted in DA causing a brief period of excitation, and whereas responses to NaCN were still

potentiated, those to ACh were slightly inhibited. There was also a tendency for spontaneous activity to increase, particularly after the 1 mg/kg dose.

Following administration of α-flupenthixol the rate of increase of chemoreceptor discharge during hypoxia was much greater than in the control, although the maximum frequency reached was not appreciably different in the two states (see Fig. 4*B*).

It has been reported that besides inhibiting the depressive action of DA in the C.N.S., α-flupenthixol also antagonizes the depressive effect of 5-HT, being only slightly more selective against DA (Straughan & Dray, 1976). Accordingly, in the present study responses to both DA and 5-HT were obtained before and after administering α-flupenthixol so that the specificity of the antagonist on chemosensory responses could be assessed. 5-HT caused a slight increase in discharge on injection followed by a period of inhibition similar to that evoked by DA (see also Nishi, 1975). There was a tendency for low doses of α-flupenthixol to reduce the excitatory action of 5-HT, although this was not consistent. The inhibitory effect was not affected until doses of 0·5 mg/kg or more were used, when the response was abolished (Fig. 2). This finding meant that results obtained following high doses of α-flupenthixol ($\geqslant$ 0·5 mg/kg, I.A.) had to be interpreted cautiously because the drug was evidently no longer acting selectively.

The dopamine antagonist *haloperidol* in cumulative doses of 0·2, 0·5 and 1·0 mg/kg, I.V. was studied in one experiment and the results obtained were similar to those obtained with α-flupenthixol. The lowest dose was without much effect, but the higher two reduced, but did not abolish, the inhibitory action of DA while potentiating the response to NaCN and inhibiting that to ACh. Spontaneous discharge frequency was 2.1 ± 0·2 c.p.s. before and 3·1 ± 0·4 after 0·5 mg/kg, and 2·1 ± 0·3 c.p.s. after the additional 1 mg/kg dose of haloperidol.

α-Methyl p-tyrosine (AMPT)

As an alternative to using receptor antagonists to interfere with the actions of endogenous DA, attempts were made to increase the turnover of DA in the presence of AMPT, a drug which blocks the biosynthesis of catecholamines by inhibiting tyrosine hydroxylase. The animals were exposed to two ten min periods of hypoxia separated by a ten min period of hyperoxia over the course of 30 min and they were then returned to breathing room air. This was done before and after administering AMPT in doses of 0·2, 1·0 and 10 mg/kg, I.A. Results from three experiments showed that the low dose slightly potentiated the responses to NaCN and hypoxia, an example of this can be seen in Fig. 4*A*; higher doses caused a further small increase. Responses to ACh were only very slightly potentiated, even after the highest dose of AMPT. Spontaneous chemoreceptor activity averaged 1·3 ± 0·1 c.p.s. before and 1·3 ± 0·2 c.p.s. after AMPT, 0·2 mg/kg. The corresponding values for 10 mg/kg, I.A. were 1·3 ± 0·1 and 1·5 ± 0·1 c.p.s. Electron microscopy revealed that depletion of dense-cored granules occurred following the 1 mg/kg dose, as compared with untreated carotid bodies exposed to alternating periods of hypoxia and hyperoxia (K. Bell, R. J. Docherty & D. S. McQueen, unpublished observations).

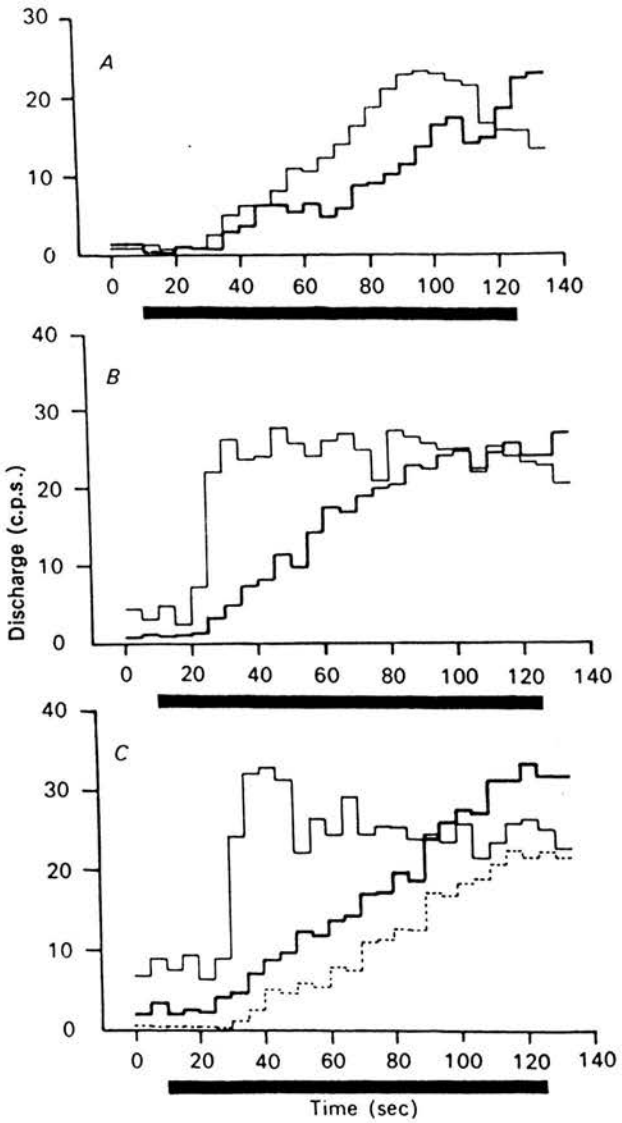


Fig. 4. The upper panel *A* shows the response of a single chemoreceptor unit (discharge averaged over 5 sec intervals) to 120 sec of hypoxia, represented by the horizontal black bar, before (——) and after (——) AMPT 0·2 (mg/kg, I.A.).

The centre panel *B* illustrates the response to hypoxia obtained from a recording containing two chemoreceptor units before (——) and after (——) administering α-flupenthixol (0·2 mg/kg, I.A.).

In the lower panel *C* the response of another two chemoreceptor units to hypoxia is shown before (——) and after (– – –) apomorphine (0·2 mg/kg, I.A.). Administration of α-flupenthixol (0·2 mg/kg, I.A.) reversed the inhibition caused by apomorphine and potentiated the response to hypoxia (——).

D-Amphetamine

Amphetamine causes release of DA (Bunney, Aghajanian, & Roth, 1973) and 10 μg was injected I.A. before and after treating the animal with AMPT in two experiments. This dose of amphetamine caused a slight inhibition of chemoreceptor discharge in the control state (although not as marked as that observed following low doses of DA) which was abolished by AMPT 10 mg/kg and hypoxia + hyperoxia.

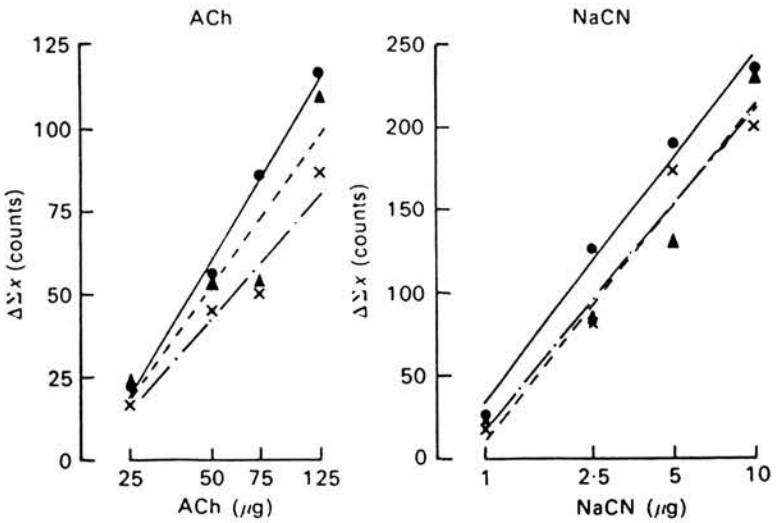


Fig. 5. Dose–response lines, from a recording of chemoreceptor activity (three units), illustrating the influence of apomorphine on responses evoked by ACh and NaCN. Doses are plotted on a $\log_{10}$ scale and straight lines, fitted to the data by the method of least squares, show: control response (●—●), responses after apomorphine, 0·05 mg/kg, I.A. (×—.—×), and after a further dose of 0·2 mg/kg, I.A. (▲---▲).

Mean control discharge frequencies were $3{\cdot}8 \pm 0{\cdot}4$ c.p.s. before apomorphine and $0{\cdot}9 \pm 0{\cdot}1$ and $1{\cdot}7 \pm 0{\cdot}2$ after 0·05 and 0·2 mg/kg apomorphine respectively.

Apomorphine

Apomorphine was used in two experiments to provide a long-lasting stimulation of the DA receptor. It was studied in cumulative doses of 0·05 and 0·2 mg/kg, I.A. and caused an inhibition of spontaneous chemoreceptor activity which persisted for up to 45 min. This allowed sufficient time to obtain dose-response data for ACh and NaCN. Although background discharge frequency was reduced, DA could still evoke an inhibitory response. Similar results were obtained in both experiments, dose–response lines from one of them being shown in Fig. 5 where it can be seen that responses to NaCN were slightly inhibited as were responses to ACh, particularly at the higher doses. The rate of increase of discharge during hypoxia was reduced following apomorphine, an effect which was reversed by α-flupenthixol (see Fig. 4*C*).

DISCUSSION

The present results confirm that DA inhibits chemosensory activity following close-arterial injection to the cat carotid body (see also Black, Comroe & Jacobs, 1972; Sampson, 1972; Zapata, 1975). This inhibitory effect of DA is primarily a direct

action on the chemoreceptors and not a consequence of vascular changes (Zapata, 1975; Sampson, Aminoff, Jaffe & Vidruk, 1976*a*; Sampson & Vidruk, 1977). Higher doses of DA caused a delayed increase in discharge which greatly reduced the duration of the inhibitory response. This increase seems similar to that observed *in vitro* by Zapata (1975) which was unaffected by dopaminergic or α-adrenergic blockers. The cause of the delayed increase was not investigated in the present study and remains to be determined.

Recently Sampson & Vidruk (1977) obtained reproducible DC potential changes with DA, even when administered every 3–4 min (dose unspecified), using the *in vitro* carotid body preparation, and they contrasted their results with Zapata's finding that frequent administration of DA reduced the chemoreceptor inhibitory response. However, whether results obtained using the mass receptor potential can be compared meaningfully with those using chemosensory discharge frequency has yet to be established. Sampson (1972) and Sampson *et al.* (1976*a*) apparently obtained repeatable inhibitory responses to DA *in vivo* using doses of 2–5 μg, which accords with our results at these doses, but they did not study the higher doses which we found gave delayed increases in discharge and inconsistent inhibitory effects.

If Osborne & Butler's theory is correct (see Introduction), chemoreceptor activity should increase when the inhibitory action of DA is blocked because spontaneous depolarization of sensory nerve endings would no longer be suppressed. Furthermore, according to their theory ACh is released from sensory nerve endings in the carotid body and acts to suppress the release of DA from Type 1 cells, thereby causing an increase in chemoreceptor discharge. In a situation where the inhibitory effect of DA has been blocked, ACh should no longer cause excitation. Our results show that blocking the inhibitory effect of DA with α-flupenthixol (0·2 mg/kg, I.A.) did not affect either spontaneous chemoreceptor activity or the response to ACh. Higher doses (e.g. $\geqslant$ 0·5 mg/kg, I.A.) did cause spontaneous activity to increase and reduced the responses to ACh, but at this dose inhibitory responses to 5-HT were also blocked, implying that the drug was not acting selectively against DA, which we assumed it was at lower doses. It should be noted that α-flupenthixol has weak α-adrenergic blocking actions (Møller Nielsen *et al.* 1973). After α-flupenthixol, 0·2 mg/kg, responses to NaCN were potentiated and the increase in discharge during hypoxia was much more rapid. Similar results were obtained with another DA antagonist, haloperidol.

In addition to the experiments with DA antagonists, we also studied the response of the chemoreceptors during prolonged DA receptor stimulation. Inhibitory responses to DA injections were short-lasting, and although the use of DA infusion to give a sustained inhibition was considered, this was rejected because of the possibility of causing secondary increases in discharge. Instead the DA agonist apomorphine (Bunney *et al.* 1973) was used and gave a long-lasting inhibition of spontaneous chemosensory activity. Responses to NaCN and hypoxia were reduced by apomorphine, as were those to ACh, but to a lesser extent. These results showed that the responsiveness of the chemoreceptors to stimulants was only slightly reduced by prolonged DA receptor stimulation. The inhibitory effect of apomorphine was prevented by α-flupenthixol, an observation which provides indirect evidence that apomorphine and DA were acting at the same site(s).

We also tried a different approach to testing the theory, the objective being to reduce endogenous DA levels in the carotid body using AMPT. Hypoxia reduces the DA content of the carotid body, both when the sinus nerve is intact (Sampson, Nicolaysen & Jaffe, 1975) and when denervated (Hellström, Hanbauer & Costa, 1976), and the presence of the tyrosine hydroxylase inhibitor AMPT (Moore & Dominic, 1971) should reduce DA biosynthesis during and after hypoxia. In our experiments AMPT was administered and the animals subjected to alternate periods of hypoxia and hyperoxia (the latter because according to Osborne & Butler's theory DA turnover should be greatest when the oxygen tension is higher than normal). The results indicated that AMPT treatment slightly potentiated the response to NaCN but had little effect on either spontaneous activity or the response to ACh. To test for DA depletion we studied the inhibitory effect of D-amphetamine, which was presumed to reflect DA release (Bunney *et al.* 1973) and which was reduced or abolished by AMPT. We also assessed, qualitatively, the reduction by AMPT in the dense-cored vesicle content of Type 1 cells, where DA is thought to be stored (Chen & Yates, 1969; Kobayashi, 1971; see also the review by Biscoe, 1971). Although Zapata *et al.* (1969) reported that prolonged hypoxia in cats had no effect on either the dense-cored vesicles or the catecholamine levels in the carotid body, more recent studies by Hellström *et al.* (1976) have shown that 15 min of hypoxia (5 % O_2 in N_2) selectively reduced the DA content in the rat carotid body.

We have interpreted the results as showing that DA levels in the carotid body were greatly reduced by the catecholamine-synthesis inhibitor AMPT, although other interpretations are possible. For example, structural changes in the Type 1 cells may reflect changes in the amount of complexing substances rather than DA content, and noradrenaline in the cat carotid body (Chiocchio *et al.* 1966) may also be affected by hypoxia (Mills & Slotkin, 1975; cf. Zapata *et al.* 1969), AMPT and amphetamine. However, responses to stimulants following the combination of AMPT and hypoxia/hyperoxia were in good agreement with results obtained using the DA antagonist α-flupenthixol, which adds further support to the interpretation that AMPT was reducing DA levels.

In summary, the present results demonstrate that responsiveness of the chemoreceptors to stimulants was only slightly affected when the dopaminergic system was influenced by blocking drugs (α-flupenthixol or haloperidol), a biosynthesis inhibitor (AMPT), or a long-acting DA agonist (apomorphine). These findings do not support the theory of Osborne & Butler (1975) that DA is tonically released in the carotid body to suppress chemosensory activity. There is also evidence in the literature which is at variance with this theory. For example, reserpine treatment does not modify the response to chemoreceptor stimulants, even though DA levels in the carotid body are substantially reduced (Eyzaguirre & Zapata, 1968; Zapata *et al.* 1969; Nishi, 1975). Also, Zapata (1975) found that the DA antagonist spiroperidol did not increase spontaneous chemosensory activity *in vitro* and he concluded that 'a continuous release of DA to maintain tonic inhibition of chemosensory fibres is not very probable'.

Our results are, however, compatible with suggestions that DA may, through its inhibitory action, *modulate* chemosensory activity (Mitchell & McDonald, 1975; Zapata, 1975). We consider that increased afferent activity causes a release of DA

which reduces the sensitivity of the sensory nerves; this does not preclude the possibility that stimulants (e.g. hypoxia) also cause a direct release of DA from storage sites. In this scheme DA release would be minimal in the normal resting state, which explains why α-flupenthixol and AMPT had no appreciable effect on spontaneous discharge frequency. Responses to ACh injections last for only 1–2 sec (not very physiological) which may not be long enough to allow any DA released by the increased activity to exert an inhibitory influence, thus explaining why responses to ACh were not greatly affected by the DA inhibitors. Responses to NaCN and to hypoxia are longer-lasting (4–10 sec for NaCN) and were evidently suppressed by DA, since they were potentiated following α-flupenthixol or AMPT. We conclude, therefore, that DA modulates an increase in chemosensory activity by influencing the rate of increase in discharge, but not the maximum discharge frequency. The mechanism whereby DA is released in response to increased chemosensory activity remains to be established, but may involve reciprocal synapses, possibly utilizing ACh (Hess & Zapata, 1972; McDonald & Mitchell, 1975), or release from Type 1 cells by increased efferent activity in the sinus nerve (Biscoe & Stehbens, 1967; Biscoe, Lall & Sampson, 1970; Neil & O'Regan, 1971; Sampson, 1972; Sampson, Aminoff, Jaffe & Vidruk, 1976*b*), although the physiological importance of the latter pathway has been questioned (McCloskey, 1975; Mitchell & McDonald, 1975).

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EFFECTS OF METHACHOLINE ON THE CAROTID CHEMORECEPTORS.

By D. S. McQUEEN. From the Department of Pharmacology, University of Edinburgh, 1, George Square, Edinburgh EH8 9JZ, Scotland.

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The present electrophysiological study shows that methacholine (MCh), generally regarded as a muscarinic agonist, stimulates the carotid chemoreceptors in pentobarbitone anaesthetized cats. The response consisted of a primary increase in discharge, attributable to nicotinic actions of MCh since it was unaffected by atropine but abolished by mecamylamine, and a delayed secondary increase in discharge, due mainly to bronchoconstriction evoked by MCh. Physostigmine caused similar potentiation of responses to acetylcholine and MCh which implies that acetylcholinesterase is located close to the site(s) at which the drugs act to stimulate chemoreceptor activity. The findings are in agreement with the general principle that chemosensory activity is increased by nicotinic agonists but not by muscarinic agonists.

It is generally accepted that the peripheral arterial chemoreceptors are stimulated by nicotinic agonists but not by muscarinic agonists [e.g. see Mercier, Rizzio and Delphaut, 1934; Philippot, 1937; Anichkov and Belen'kii, 1963]. There is, however, disagreement concerning the chemoreceptor-stimulating properties of the muscarinic agonist methacholine (acetyl β methylcholine; MCh), a drug reported to be virtually devoid of nicotinic actions [Simonart, 1932; Hunt and Renshaw, 1934]. From studies based on reflex respiratory changes in dogs De Wispeleare [1937a, b] claimed that MCh stimulates carotid chemoreceptors, while in contrast Comroe and Starr [1933] found that MCh did not stimulate the chemoreceptors in cats, this being confirmed by Philippot [1937] and Comroe and Schmidt [1938] in dogs. The electrophysiological investigation performed by Liljestrand and Zotterman [1954] does not clarify the matter since they concluded that large doses of MCh stimulated the chemoreceptors in cats, but noted that the hypotension associated with MCh made it difficult to interpret the results.

In view of these discordant results, it was decided that a quantitative electrophysiological investigation should be performed in order to establish whether or not MCh stimulates the cat carotid chemoreceptors.

METHODS

Experiments were performed on 7 cats, mean weight 3·0 kg, range 2·3–4·5 kg. Full details of the experimental procedures have been described previously [McQueen, 1977], and only a brief summary follows. The animals were anaesthetized with pentobarbitone sodium, (42 mg.kg^{-1} i.p.), artificially ventilated with air and paralysed by gallamine 3 mg.kg^{-1} i.v.). Blood pressure was recorded from a femoral artery and the lingual artery ipsilateral to the sinus nerve from which recordings were obtained was cannulated, the tip of the cannula being in the common carotid artery 2 cm caudal to the carotid bifurcation. The bladder was drained at regular intervals and rectal temperature maintained at 38 ± 0·5°C.

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The sinus nerve was sectioned central to its junction with the glossopharyngeal nerve. Electrical activity from single or multiple chemoreceptor units was recorded from filaments of the peripheral nerve using bipolar platinum-iridium electrodes and an A.C. amplifier (Neurolog, Digitimer). Chemoreceptor units were identified by their random discharge [Eyzaguirre and Lewin, 1961; Biscoe and Taylor, 1963] and their increase in discharge frequency following injection of NaCN (0·1 μmol) into the ipsilateral common carotid artery. The ganglio-glomerular nerves were cut in order to eliminate reflex effects of sympathetic activity on carotid nerve discharge [Floyd and Neil, 1952; Eyzaguirre and Lewin, 1961]. Nerve activity was recorded on tape (Tandberg 100, frequency response d.c.–1250 Hz) and subsequently replayed through a pulse height discriminator linked to a PDP–8 computer. The average ($\bar{x}$) and total count (Σx) were calculated for each response after its duration (t s) had been determined. A 'response' was defined as being from the first substantial (i.e. 3 times or more) increase above the mean control discharge frequency until the discharge returned to pre-injection level. Responses were expressed as an increment above control level by subtracting the appropriate control value, i.e. as $\Delta\bar{x}$ and $\Delta\Sigma x$.

The effect of MCh was determined by injecting 0·1 ml of drug solution into the common carotid artery *via* the lingual catheter and washing it in with 0·2 ml Locke solution. Injections were made over 2 s and were repeated every 15 min. Other drugs were injected i.v. or intra-carotid over 5–10 s and 20 min allowed before retesting MCh. The catheter dead-space was 0·1 ml.

Drugs. Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42g; $CaCl_2$ 0·24 g; Tris base 6·0 g; N HCl 39 ml; distilled water to 1 l; pH 7·41 at 37°C.)

The drugs used were: pentobarbitone sodium (Abbot Laboratories); gallamine triethiodide (May and Baker); acetylcholine iodide, sodium cyanide, physostigmine salicylate, carbamoylcholine chloride (carbachol), atropine sulphate—all B.D.H.; acetyl β methylcholine bromide (MCh)—Koch Light.

Results

Primary and secondary responses

In each of the seven experiments in which it was studied MCh evoked a dose-dependent increase in chemoreceptor discharge when injected i.a. The response could be divided into a primary and a secondary component. The primary response was intense and commenced immediately following the injection but it only lasted for 1–2 s. It was followed by a 5–10 s period of relative inhibition before a sustained discharge gradually arose, this secondary increase being associated with a fall in mean B.P. and a rise in end-tidal CO_2 (see Figs. 1 and 2A). MCh was 12–15 times less potent than acetylcholine (ACh) in its primary chemoreceptor-stimulating ability (see Fig. 3), a similar ratio being obtained when $\Delta\bar{x}$ rather than $\Delta\Sigma x$ data were used. The secondary increase was not seen with ACh, even though B.P. fell.

Atropine

This muscarinic antagonist was administered in four experiments. The primary response was not appreciably affected by 1 mg.kg^{-1} i.v. (see Figs. 1 and 2B) whereas the secondary increase was reduced. B.P. still fell quite low, this being in agreement with reports by Simonart [1932] and Hunt and Renshaw [1934] that the vascular effects of high doses of MCh cannot be blocked by

atropine, even in high doses. There was only a slight increase in end-tidal CO_2. Atropine was also administered close-arterial to the carotid body (1 mg.kg^{-1} i.a.) in three of these experiments, but both the primary and secondary responses evoked were very similar to those observed after i.v. atropine.

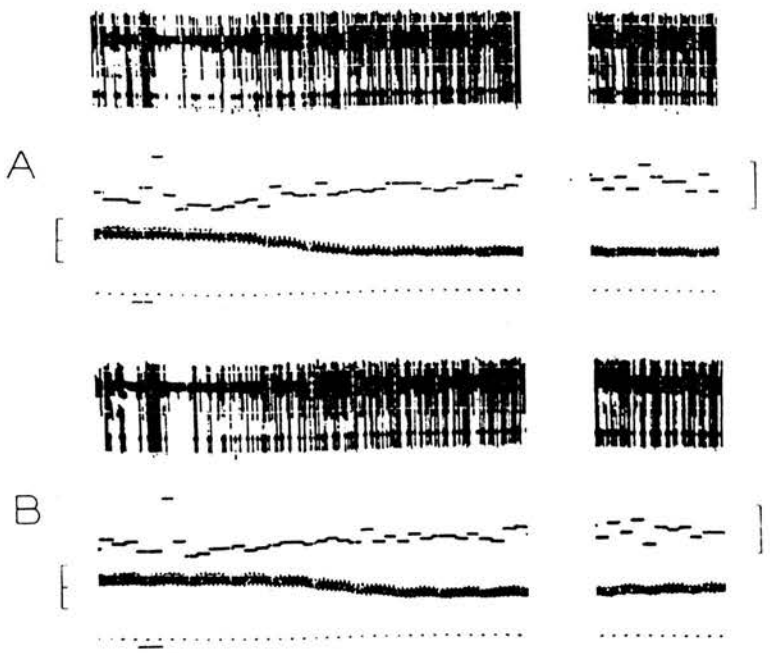

FIG. 1. Recording of chemoreceptor activity showing the response evoked by MCh (2·08 μmol i.a.) before (A) and after (B) atropine (1 mg.kg^{-1} i.v.). The secondary response can be seen in the second panel where the discharge starting 35 s after the injection is shown. Primary responses are presented in more detail in Fig. 2 (A and B).

Panels show from above downwards: nerve action potentials, the pulse height discriminator being used to count a single unit at the level indicated by the brightening pulse; counter output, which had been stored for 1 s, in counts. s^{-1}, calibration on right of figure: 0–20 counts. s^{-1}; blood pressure, calibration on left of figure: 0–100–200 mm Hg; 1 sec time marker; injection marker.

Physostigmine (*Eserine*)

In two experiments the influence of physostigmine (1·2 mg.kg^{-1} i.a.) on the response to MCh was studied in the atropinized cat and it was found that the primary response was greatly potentiated (see Fig. 2C). Responses to concomitantly administered ACh were also potentiated, as shown by data from one of the experiments illustrated in Fig. 3. The control discharge was not affected by physostigmine.

The assumption was made that the potentiation of responses to MCh and

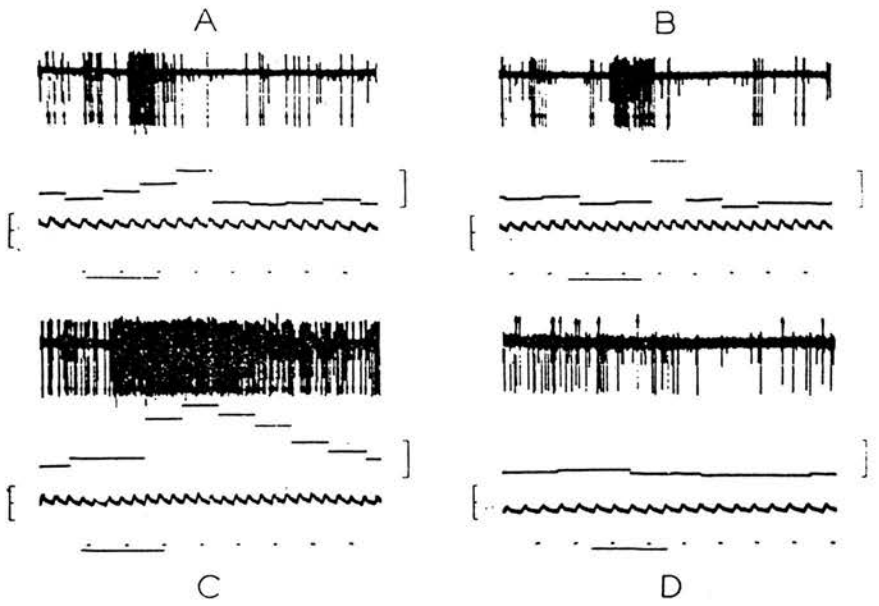

FIG. 2. Same recording as Fig. 1 showing the response to MCh (2·08 μmol i.a.) (A) before and (B) after atropine (1 mg.kg^{-1} i.v.), (C) after physostigmine (1·2 mg.kg^{-1} i.a.), and (D) after mecamylamine (1 mg.kg^{-1} i.a.).

Record details as for Fig. 1. In (D) the single unit was counted from the positive wave of its action potential.

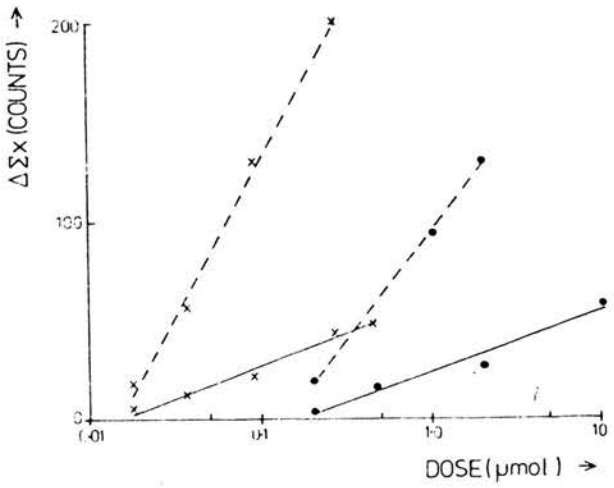

FIG. 3 Dose response data from a single unit showing the effects of MCh (●) and ACh (X) before (——) and after (– – –) physostigmine (1·2 mg.kg^{-1} i.a.) in an atropinized (1 mg.kg^{-1} i.v.) cat. Straight lines were fitted to the data by the method of least squares. The dose is plotted on a $\log_{10}$ scale against $\Delta\Sigma x$, the increase above control level of the integrated discharge.

ACh resulted from a specific anticholinesterase action of physostigmine. This was justified by the observation that responses to carbachol, a stimulant not destroyed by cholinesterase, and NaCN were unaltered after administering physostigmine, thereby making it unlikely that the anticholinesterase was acting in some non-specific manner (e.g. to affect blood flow or sensory nerves in the carotid body).

Mecamylamine

In three experiments the nicotinic antagonist mecamylamine was administered (1 $mg.kg^{-1}$ i.a.) in the atropinized preparation, in two of which physostigmine had previously been given. Mecamylamine completely abolished the primary response to MCh, even when doses as high as 10 μmol of the agonist were given. The effect lasted for several hours, but was reversible. During the time the preparation was refractory to MCh and ACh, it continued to respond normally to NaCN.

Discussion

The present experiments demonstrate that MCh stimulates the carotid chemoreceptors causing a brief primary excitation followed by a delayed secondary increase in discharge. Since the *primary* excitation was unaffected by atropine, but was completely abolished by mecamylamine, it seems reasonable to conclude that it is due to a nicotinic action of MCh. The secondary response was associated with a fall in B.P. and a rise in end-tidal CO_2, the latter being attributable to the potent broncho-constricting action of MCh [De Wispeleare, 1937b]. Atropine reduced the secondary response by preventing much of the bronchoconstriction; blood pressure still fell, thereby showing that the greater part of the *secondary* excitation observed before atropine resulted from bronchoconstriction and not hypotension.

It is difficult to compare existing reports concerning the actions of MCh on carotid chemoreceptors because very little evidence is presented to support the conclusions. De Wispeleare [1937a, b] showed that MCh given i.v. stimulates the chemoreceptors in dogs. One might argue that part of the hyperpnoea he observed may have been due to increased chemoreceptor activity secondary to the reflex increase in sympathetic tone to the carotid body that would be evoked by hypotension [Floyd and Neil, 1952; Eyzaguirre and Lewin, 1961; Biscoe and Purves, 1967]. Nevertheless, MCh can be seen to have increased respiration *before* B.P. fell (see Fig. 13 in 1937b paper), so at least part of the effect may have been due to a direct action on the chemoreceptors. On the other hand, Comroe and Starr [1933] using cats, and Philippot [1937] and Comroe and Schmidt [1938] using dogs, all found that MCh did not possess any primary chemoreceptor-stimulating action. No evidence is given, so one can only speculate that the failure to demonstrate the chemoreceptor-stimulating action of MCh (i.a.) may have been a consequence of the use of reflex respira-

tory changes as the indicator of chemosensory activity. The respiratory response elicited would be determined by the phase of the respiratory cycle at the moment of injection [Black and Torrance, 1967], and a short-lasting response, such as MCh evokes, could easily have been missed. Comroe and Starr [1933] did note a delayed change in respiration following i.v. MCh which they concluded was secondary to the hypotension. Liljestrand and Zotterman [1954] undertook what appears to have been the only electrophysiological investigation of the actions of MCh on chemoreceptors, but again no evidence is presented to support their assertion that MCh stimulated the chemoreceptors, but only when high doses (at least ten times greater than ACh) were administered, and they commented that the hypotension made it difficult to interpret the results.

During this study the influence of physostigmine on the chemoreceptor-stimulating action of MCh was examined. Physostigmine inhibits the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE, pseudocholinesterase), both of which are present in low concentrations in the cat carotid body, BuChE being predominant [Hollinshead and Sawyer, 1945; Koelle, 1950; Biscoe and Silver, 1966]. Knowing that ACh is a substrate for both enzymes whereas MCh is only hydrolyzed by AChE (see Koelle and Gilman [1949] for a review on anticholinesterases), the finding that responses to MCh and ACh were potentiated to a similar extent by physostigmine (see Fig. 3) suggests that both stimulants were being inactivated mainly be AChE. This implies that AChE is located close to the site at which the drugs were acting to increase chemoreceptor activity. Koelle [1951] and Biscoe and Silver [1966] found BuChE and AChE were similarly distributed in the cat carotid body whereas Ballard and Jones [1971], using electron microscopic techniques, reported that AChE is localized to peripheral axons, and BuChE to the Type II cells. However, as discussed by Jones [1975], conflicting evidence and problems with the methodology mean that at present it is difficult to be certain about the localization of AChE in the carotid body.

In conclusion, MCh does stimulate the cat carotid chemoreceptors and the primary excitation is associated with nicotinic properties of the drug, this being in accord with the principle referred to in the introduction that chemoreceptors are stimulated by nicotinic agonists but not by muscarinic agonists. The generalization that MCh is virtually devoid of nicotinic actions may well be correct when the effects of the drug on, say, autonomic ganglia are being considered because the higher concentrations of AChE present there would rapidly inactivate the stimulant [Sawyer and Hollinshead, 1945; Ord and Thompson, 1950]. However, at a site such as the carotid body where AChE levels are low, the nicotinic action is obtained and the generalization is invalid.

Acknowledgments

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THE EFFECTS OF ACETYLCHOLINE AND DOPAMINE ON CAROTID CHEMOSENSORY ACTIVITY IN THE RABBIT

BY R. J. DOCHERTY AND D. S. McQUEEN
From the Department of Pharmacology, University of Edinburgh, 1, George Square, Edinburgh EH8 9JZ

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SUMMARY

1. Intracarotid (I.C.) injection of either acetylcholine (ACh) or dopamine inhibited spontaneous chemosensory activity recorded from the peripheral cut end of the sinus nerve in the anaesthetized rabbit.

2. High doses of ACh ($\geqslant$ 50 μg I.C.) evoked a slight increase in discharge which preceded the inhibition. This excitation was attributable to a nicotinic action of the drug since it was abolished by mecamylamine.

3. The muscarinic agonist bethanechol inhibited chemoreceptor activity, an effect which was blocked by *high* doses of atropine, as was the inhibition caused by ACh. Dopamine-induced inhibition was unaffected by atropine.

4. Atropine, in doses sufficient to abolish the vasodepressor effect of ACh, only slightly reduced the inhibitory action of ACh on the chemoreceptors. Also, the vasodilators sodium nitrite and sodium nitroprusside did not appreciably alter chemosensory discharge. It seems unlikely, therefore, that the inhibitory response to ACh is secondary to vascular changes.

5. The inhibitory response to dopamine, but not that to ACh, was blocked by the dopamine antagonist α-flupenthixol. This implies that inhibition of chemosensory activity evoked by exogenous ACh was not secondary to dopamine release.

6. The implications of the results are discussed, particularly with regard to the possible physiological role of ACh as a modulator of carotid chemosensory activity.

INTRODUCTION

The present electrophysiological investigation was undertaken to examine the effects of acetylcholine (ACh) and dopamine on carotid chemosensory activity in the rabbit. It is well known that ACh stimulates arterial chemoreceptors in the cat (e.g. see Anichkov & Belen'kii, 1963; Schweitzer & Wright, 1938) and dog (e.g. Heymans, Bouckaert, Farber & Hsu, 1936), but there do not appear to be any reports concerning the effects of ACh, or other drugs which affect the cholinergic system, on the activity of rabbit carotid body chemoreceptors.

Dopamine seems to have species-dependent effects on the carotid chemoreceptors, stimulating sensory activity in dogs (Jacobs & Comroe, 1968), but inhibiting it in cats (Black, Comroe & Jacobs, 1972; Sampson, 1972; Zapata, 1975; Docherty & McQueen, 1978*a*). We were interested in determining what effect dopamine has on chemosen-

sory activity in the rabbit, particularly since it is present in the carotid body of this species (Dearnaley, Fillenz & Woods, 1968). A preliminary account of some of this work has been presented to the Physiological Society (Docherty & McQueen, 1978*b*).

METHODS

Experiments were performed on male rabbits (New Zealand White or Californian) weighing between 2·5 and 3·5 kg (mean weight = 2·9 kg).

Anaesthesia. Animals were anaesthetized with sodium pentobarbitone (30–50 mg/kg) or urethane (400 mg/kg) and α-chloralose (6 ml./kg of a 1% solution in saline), administered through an ear vein, with supplements as required (Korner, Uther & White, 1968).

General. A cannula was inserted in the trachea low in the neck. Blood pressure was measured via a pressure transducer (Bell & Howell, 4-422) from a cannulated femoral artery, displayed on a pen recorder (Devices, M4), and recorded by an FM tape recorder (Tandberg, 100; frequency response d.c. to 1250 Hz). Arterial blood pH, P_{o_2}, and P_{co_2} were measured at hourly intervals using a Radiometer gas monitor (BMS 3 with PHM 71 meter). A femoral vein was cannulated and used for drug administration. Rectal temperature was monitored and maintained at 39 ± 0·5 °C by a heating pad.

The carotid bifurcation region was exposed and dissected free from surrounding tissue. A cannula was inserted into the lingual artery and advanced until its tip lay in the common carotid artery approximately 1·5 cm caudal to the carotid bifurcation. This cannula was used for close-arterial administration of drugs to the cartoid body.

Recording sinus nerve activity. The animal was artificially ventilated with room air by a respiratory pump (S.R.I.) operating at 38 strokes/min and gallamine triethiodide (3 mg/kg I.V.) was administered to paralyse spontaneous respiration. This dose had no appreciable effect on chemoreceptor responses to ACh or dopamine. In most experiments end-tidal CO_2 was continuously monitored by an infra-red CO_2 analyser (Med 1A; Grubb Parsons) and the stroke volume of the pump adjusted to maintain end-tidal CO_2 at 5%. The carotid sinus nerve ipsilateral to the catheterized lingual artery was cut centrally and activity in the nerve was recorded as described previously (McQueen, 1977). Chemoreceptor units were identified by their random pattern of discharge, their increase in discharge frequency following injection of sodium cyanide (5 μg) into the ipsilateral common carotid artery, their increase in discharge in response to hypoxia (breathing 5% oxygen in nitrogen), and by the inhibition of discharge in response to hyperoxia (breathing 100% oxygen).

Drug administration. Intracarotid (I.C.) injections of drugs were made in a volume of 0·1 ml. and the catheter (dead space = 0·1 ml.) flushed with 0·2 ml. modified Locke solution at 37 °C which had been bubbled with a 5% carbon dioxide/95% air gas mixture. Injections were made at the peak of the inspiratory phase of the respiratory cycle and completed over one respiratory cycle. I.V. injections of drugs were made in a volume of 0·2–1·0 ml. and the catheter flushed with 0·5 ml. saline. In experiments with mecamylamine, the appropriate volume of dextran solution (2·5% dextran, 5% glucose in distilled water) required to maintain blood pressure at the control level, was administered I.V. This treatment prevented the sustained fall in blood pressure which would otherwise have accompanied administration of this ganglion blocking drug.

Data analysis. Action potentials were counted and data analysed as previously described (McQueen, 1977). Responses to drugs were expressed as the absolute difference in discharge following drug administration where

$$\Delta\Sigma x = \Sigma x \text{ (response)} - \Sigma x \text{ (control)}$$

and

$$\Sigma x \text{ (control)} = \bar{x} \text{ (control)} \times t,$$

$\Delta\Sigma x$ = absolute difference in discharge,

Σx = total discharge,

$\bar{x}$ = average discharge (counts per second),

t = response duration (sec).

Drugs. Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42 g; $CaCl_2$ 0·24 g; Tris base 6·0 g; N-HCl 39 ml.; distilled water to 1 l.; pH = 7·41 at 37 °C), excepting α-flupen-

thixol which was prepared in 0·9% (w/v) sodium chloride solution. Doses referred to are those of the salts.

The drugs used in this investigation were: pentobarbitone sodium, gallamine triethiodide (May & Baker); acetylcholine iodide, sodium cyanide, atropine sulphate; urethane (ethyl carbamate), α-chloralose, sodium nitrite (B.D.H.); bethanechol chloride, dopamine hydrochloride (Koch-Light); sodium nitroprusside (Griffin & Tatlock); mecamylamine hydrochloride (M.S.D.); 5-hydroxytryptamine creatinine sulphate (Labkemi A. B.); suberyldicholine di-iodide (kindly supplied by Dr A. Ungar, Department of Pharmacology, University of Edinburgh).

RESULTS

Experiments were performed on twenty-one rabbits from which twenty-three recordings (three single and twenty multiple units) of chemoreceptor activity were obtained.

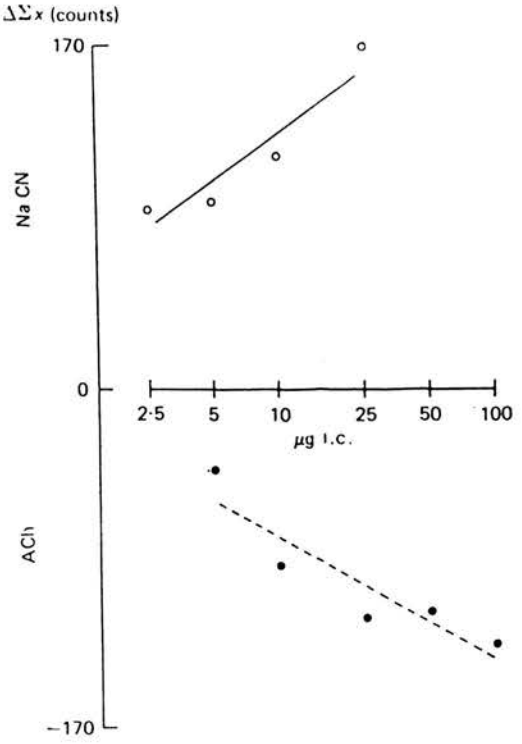

Fig. 1. Dose–response data for sodium cyanide, NaCN (○—○) and acetylcholine, ACh (●---●) in one experiment (three chemoreceptor units). In this and subsequent Figures doses (μg I.C.) are plotted on a $\log_{10}$ scale and responses expressed as $\Delta\Sigma x$ unless otherwise indicated (see Methods section). Lines were fitted to the data by the method of least squares.

Responses to cholinergic agonists

Acetylcholine. Injections of ACh (1–250 μg I.C.) caused an immediate, short-lasting (1–30 sec) inhibition of chemoreceptor activity in all the experiments, including two in which the ipsilateral superior cervical ganglion had been removed. There was an approximately linear relationship between $\log_{10}$ dose and response ($\Delta\Sigma x$) over the range 5–100 μg I.C. (see Fig. 1). Doses in this range, which always caused inhibition

of chemoreceptor activity in the rabbit (either anaesthetic), are comparable to doses which stimulate chemoreceptor activity in the cat (McQueen, 1977). With high doses of ACh ($\geqslant$ 50 μg I.C.), the inhibition was preceded by a slight (2–3 times control discharge), transient period of stimulation lasting less than 1 sec (see Fig. 2).

Mecamylamine. Mecamylamine is a potent ganglion blocking drug (Stone, Torchiana, Navarro & Beyer, 1956; Bennet, Tyler & Zaimis, 1957), which can block the increase in chemoreceptor activity evoked by ACh in the cat carotid body, both *in vitro* (Eyzaguirre & Zapata, 1968) and *in vivo* (Sampson, 1971; McQueen, 1977).

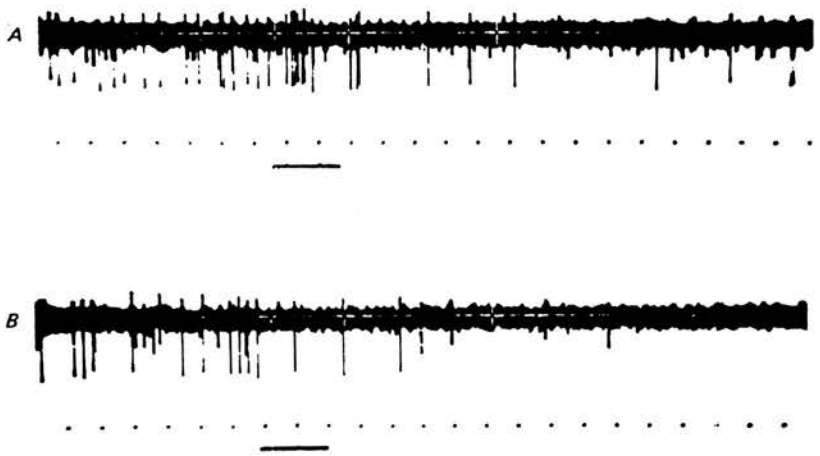

Fig. 2. Chemoreceptor units from an experiment showing the early part of the response to a high dose of ACh (50 μg I.C.), before (*A*) and after (*B*) administration of mecamylamine (0·5 mg/kg I.C.). Panels show from above downwards: action potentials, 1 sec time markers, injection marker.

The inhibition of chemoreceptor activity evoked by ACh in rabbits was either unaffected or potentiated following the administration of mecamylamine (1–5 mg/kg I.V. or 0·25–0·5 mg/kg I.C.). The transient stimulation seen with high doess of ACh was, however, abolished (Fig. 2).

Suberyldicholine (SDCh). SDCh is a potent nicotinic agonist which has been shown to stimulate the carotid chemoreceptors of the cat (Anichkov & Belen'kii, 1963; Dardymov & Ger, 1964; McQueen, 1974). Intracarotid injection of SDCh (10–50 μg) in the rabbit had no consistent effect on carotid chemoreceptor activity.

Atropine. Atropine, in doses (1–10 mg/kg I.V.) sufficient to block the depressor effects of ACh, caused only a slight reduction in the inhibition of chemoreceptor activity produced by ACh (see Fig. 3). There was a large variation in the dose of atropine required to block the vascular effects of ACh in different animals. However, administration of atropine close-arterial to the carotid body (1 mg/kg I.C.) caused a substantial reduction in the chemoreceptor response to ACh (Fig. 4*A*).

Bethanechol. Bethanechol is a muscarinic agonist which is relatively free of nicotinic actions (Molitor, 1936). I.C. injection of bethanechol (10–100 μg) produced a dose-related inhibition of chemoreceptor activity which was greatly reduced by administration of atropine (1 mg/kg I.C.; see Fig. 4*B*). Unlike the response to ACh, the inhibition was not preceded by a stimulant effect at high doses.

Responses to sodium cyanide

Sodium cyanide increased carotid chemosensory activity in all the experiments, including the two in which the ipsilateral superior cervical ganglion had been extirpated. The threshold dose of stimulation was about 2·5 μg I.C. and a maximum response was elicited by 25 μg I.C. The effective dose range is comparable to that for stimulation of carotid chemoreceptors in the cat (McQueen, 1977). Fig. 1 shows

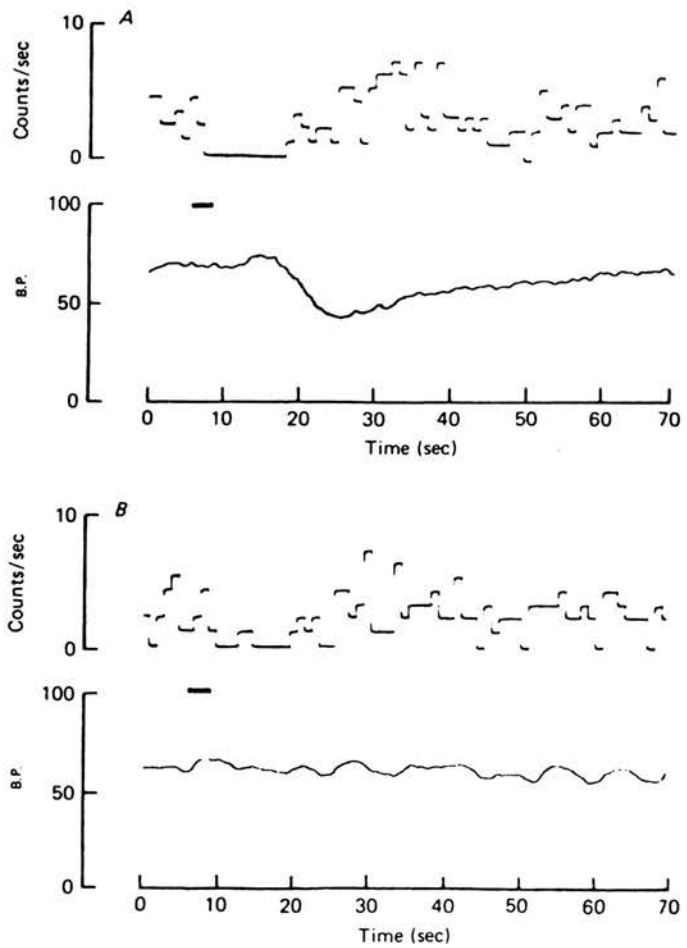

Fig. 3. Response of carotid chemoreceptors (three units) to ACh (10 μg I.C.) before (*A*) and after (*B*) administration of atropine (10 mg/kg I.V.). Panels show from above downwards: discharge frequency (counts/sec), injection marker, mean blood pressure (mmHg), time (sec).

dose–response data for sodium cyanide, illustrating the approximately linear relationship between $\log_{10}$ dose and the response ($\Delta\Sigma x$) over this range.

The chemoreceptor response to sodium cyanide was not appreciably affected by administration of either atropine or mecamylamine, although in some experiments

there was a slight reduction in the response to sodium cyanide in the presence of mecamylamine (see Figs. 5*A*, *B*).

Response to dopamine and 5-hydroxytryptamine

Dopamine. The effect of dopamine on carotid chemoreceptor activity was studied in sixteen experiments. A single injection (5–10 μg I.C.) caused an immediate, short-lasting (5–20 sec) inhibition of chemoreceptor activity in every experiment (see Fig. 7). The inhibition was sometimes followed by excitation, a phenomenon which

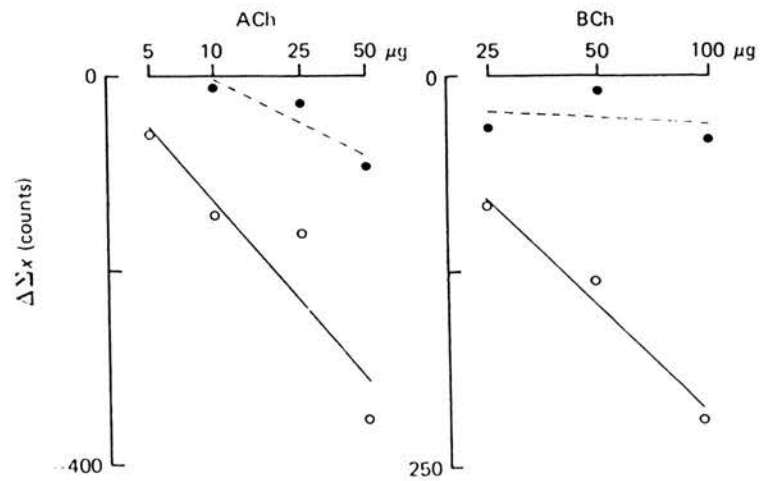

Fig. 4. Dose–response data from one experiment (three units) for ACh and bethanechol (BCh), before (○—○) and after (●---●) atropine (1 mg/kg I.C.).

has also been observed in cats (Zapata, 1975, 1977; Docherty & McQueen, 1978*a*). The chemoreceptor response to dopamine was unaltered by mecamylamine (0·5–5 mg/kg I.V. or 0·25–0·5 mg/kg I.C.) or atropine (1–5 mg/kg I.V. or 1 mg/kg I.C.).

α-Flupenthixol. α-Flupenthixol is a potent dopamine antagonist (Møller Nielsen, Pederson, Nymark, Franck, Boeck, Fjalland & Christensen, 1973) which has been shown to block the inhibitory action of dopamine on carotid chemosensory discharge in the cat (Docherty & McQueen, 1978*a*). Administration of α-flupenthixol (0·25–1 mg/kg I.V. or 0·25–0·5 mg/kg I.C.) in rabbits blocked the inhibitory action of dopamine on carotid chemosensory activity (see Fig. 6*A*) but did not reduce the inhibitory response to ACh (see Fig. 6*B*). The excitatory response to sodium cyanide was potentiated by α-flupenthixol (Fig. 5*C*).

5-Hydroxytryptamine (5-HT). The effect of 5-HT on carotid chemosensory activity was studied in eight experiments. Intracarotid injection of 5-HT (5–10 μg) caused an intense but transient stimulation of chemoreceptor activity followed by a period of relative inhibition (5–15 sec; see Fig. 7). Both the excitatory and inhibitory components of the response were subject to considerable variation in any experiment, making an accurate assessment of the effects of blocking drugs difficult. The response did not appear to be modified by either mecamylamine or atropine. Low doses of α-flupenthixol (0·25–0·5 mg/kg I.V.) had little effect on the chemoreceptor response to 5-HT although responses to dopamine were blocked. Higher doses of α-flupenthixol

(0·25–0·5 mg/kg I.C.) blocked the inhibitory component of the response to 5-HT, this being similar to the block of 5-HT inhibition observed in cats following higher doses of α-flupenthixol (Docherty & McQueen, 1978*a*).

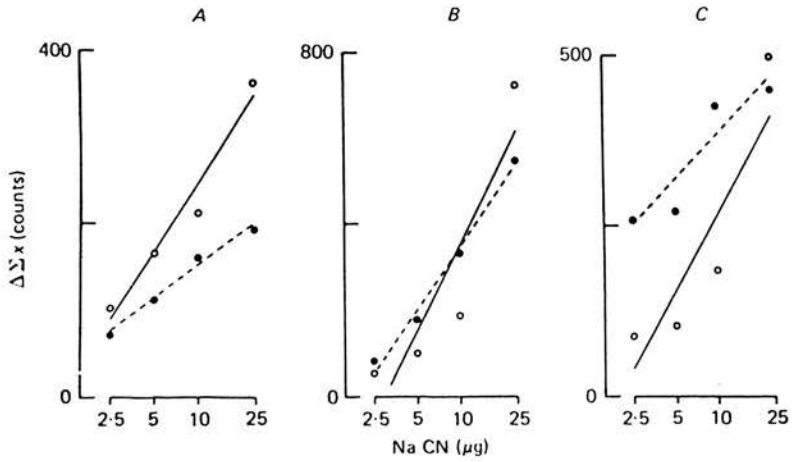

Fig. 5. Dose–response data for NaCN, obtained during three experiments, before (○—○) and after (●---●), *A*, mecamylamine (0·25 mg/kg I.C.), *B*, atropine (2 mg/kg I.V.) and, *C*, α-flupenthixol (0·25 mg/kg I.V.). Data were from recordings of three, four and three chemoreceptor units respectively.

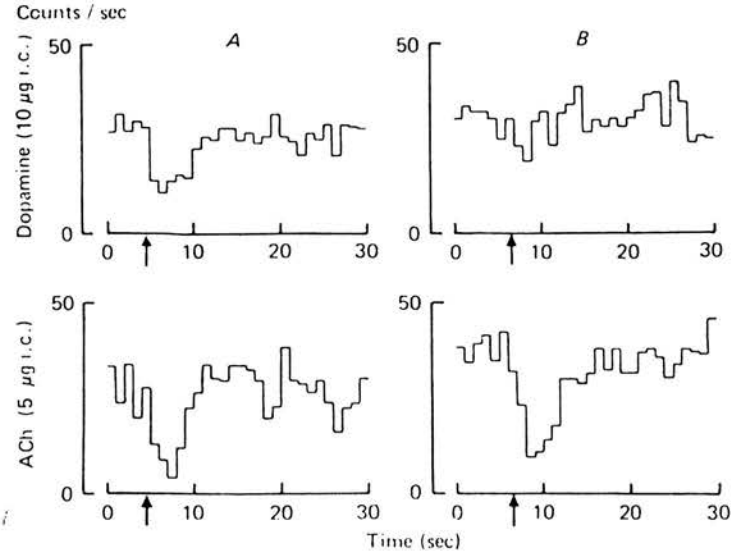

Fig. 6. Effects of dopamine (10 μg I.C.) and ACh (5 μg I.C.) on spontaneous chemoreceptor discharge (counts/sec), before (*A*) and after (*B*) administration of α-flupenthixol (0·5 mg/kg I.V.). Injections of ACh or dopamine were made at the arrows. Panels show from above downwards: discharge frequency (counts/sec), time (sec).

Response to vasodilator substances

Sodium nitrite. Sodium nitrite is a potent vasodilator which acts directly on vascular smooth muscle (Weiss, Wilkins & Haynes, 1937). Injection of sodium nitrite (25–100 μg I.C.) evoked a very slight inhibition of chemoreceptor activity lasting 3–4 sec, but the magnitude of the inhibition did not appear to be dose-related and was no greater than that caused by I.C. injection of an equal volume of modified Locke solution (0·1 ml.).

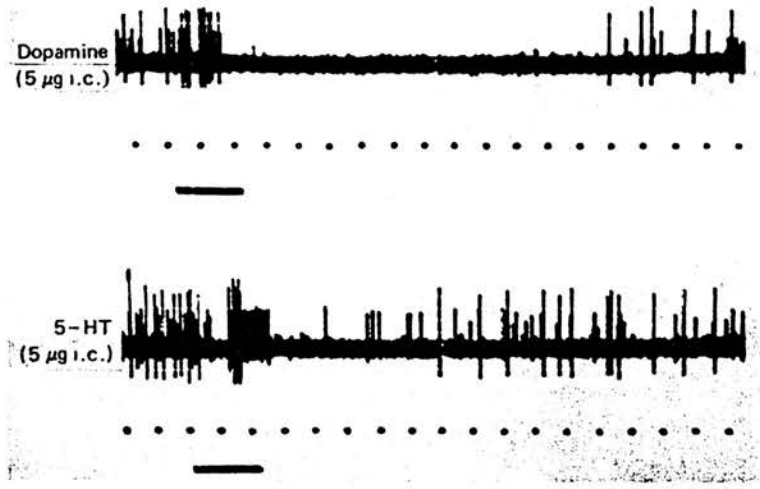

Fig. 7. Chemoreceptor units from an experiment showing the response to dopamine (5 μg I.C.) and 5-hydroxytryptamine (5-HT) (5 μg I.C.). Details as for Fig. 2.

Sodium nitroprusside. Like sodium nitrite, sodium nitroprusside is a vasodilator which acts directly on vascular smooth muscle (Johnson, 1929). Sodium nitroprusside (10–50 μg I.C.) evoked a feeble, short lasting inhibition of chemoreceptor activity, independent of dose, followed by a secondary stimulation which was dose-dependent. The magnitude of the initial inhibition evoked by sodium nitroprusside was no greater than that caused by a control injection of an equal volume (0·1 ml.) of modified Locke solution.

DISCUSSION

The results show that ACh and dopamine both inhibit spontaneous carotid chemosensory activity in anaesthetized rabbits. The inhibitory response to ACh was unexpected, being in complete contrast to the stimulant action obtained in other species (see Introduction). Experiments were performed to investigate the mechanisms whereby the drugs cause inhibition of chemoreceptor activity. The results obtained are discussed below.

Vascular effects. Inhibition of chemoreceptor discharge could have resulted from an action of ACh on vascular smooth muscle, although the short latency to onset of the effect argues against this, at least for the early part of the response. Vasodilatation of the carotid body vasculature would increase blood flow into the glomus and might thereby be expected to reduce spontaneous chemosensory discharge frequency before

any tendency for discharge to increase secondary to the delayed fall in B.P. caused by circulation of the ACh (see Fig. 3), although autoregulation of blood flow that can occur in the rabbit carotid body (McCloskey, 1968) complicates the speculation. The results from our experiments show that whereas a low dose of atropine blocked the ACh-induced fall in systemic B.P., the inhibitory response of the chemoreceptors to ACh was only slightly reduced. The vascular explanation therefore seemed unlikely, providing that the carotid body vascular responsiveness to ACh is similar to that of the peripheral vasculature. This may or may not be the case. However, the potent vasodilators sodium nitrite and sodium nitroprusside also had little effect on chemosensory activity when administered close-arterial to the carotid body, the increase in discharge associated with nitroprusside probably being due to the production of cyanide ions during the conversion of nitroprusside to thiocyanate. These results with atropine and the vasodilator drugs indicate that although vasodilatation may explain a small part of the inhibition of chemoreceptor activity evoked by ACh, it is not responsible for the greater part of the effect. This view is supported by the recent finding that ACh inhibits chemosensory activity of the superfused rabbit carotid body *in vitro* (Monti-Bloch & Eyzaguirre, 1977), this being a preparation in which vascular effects are precluded.

Characterization of the cholinergic receptors. We attempted to identify the cholinergic receptors responsible for the effects of ACh. High doses of ACh caused a transient slight stimulation of the chemoreceptors, an effect which preceded the inhibition. This excitation appeared to be attributable to a *nicotinic* action of ACh since it was abolished by mecamylamine. We are unable to explain why the nicotinic agonist SDCh was inconsistent in its ability to stimulate the chemoreceptors. In any event, as the excitatory effect was slight and only seen following injection of high doses of ACh, it is unlikely to have much physiological significance.

The inhibitory action of ACh was evidently mediated by a *muscarinic* mechanism because the muscarinic agonist bethanechol, but not the nicotinic SDCh, also caused inhibition, and the response could be blocked by the muscarinic antagonist atropine, although relatively high doses were needed. Interpretation of data derived from experiments involving the use of atropine in rabbits is complicated by the presence of an atropinase enzyme in some rabbits (Ambache, 1955), a fact which may account for the large variation in the dose of atropine required to prevent the vascular effects of ACh. Nevertheless, it was possible to block the vasodepressor action of ACh with doses of atropine which were lower than those required to block the chemoreceptor inhibitory response.

The possibility exists that the muscarinic receptors mediating the inhibition of chemosensory activity evoked by ACh are 'atypical', perhaps being similar to those in the adrenal medulla (Henderson & Ungar, 1977), or in sympathetic ganglia (Hilton, 1977), which are also fairly resistant to atropine blockade. Further studies are required to investigate this possibility.

ACh-dopamine interaction. It has been suggested that ACh may release dopamine from the rat carotid body via a muscarinic mechanism (Hellström, Hanbauer & Costa, 1976). Since we found that dopamine inhibited rabbit chemosensory activity, and it is known to be present in the rabbit carotid body (Dearnaley *et al.* 1968), the possibility that ACh-induced inhibition was secondary to dopamine release became

attractive. However, it is unlikely to be the case because the dopamine antagonist α-flupenthixol, in doses sufficient to block the inhibitory response to exogenous dopamine, did not reduce the inhibitory response evoked by ACh.

Although we found that dopamine inhibited carotid chemoreceptor activity in the rabbit, as it does in the cat (Docherty & McQueen, 1978*a*), it should be noted that Monti-Bloch & Eyzaguirre (1977) reported that it *increases* chemoreceptor discharge of the superfused rabbit carotid body *in vitro*. We found there was a delayed excitation following some doses of dopamine, but this effect was invariably preceded by an inhibition of chemosensory activity. A possible explanation for the difference between the *in vivo* and the *in vitro* results is that vascular effects are responsible for the inhibition observed *in vivo*; such vascular changes should not occur in the superfused preparation. Sampson, Aminoff, Jaffe & Vidruk (1976*a*) presented evidence showing that vascular effects are unlikely to account for the dopamine-induced inhibition of chemosensory activity in cats; whether a similar situation pertains in the rabbit remains to be established. However, we consider it unlikely that the inhibition observed *in vivo* is secondary to vascular effects of dopamine, particularly since the latency to onset of effect following the start of an injection is so short (1–2 sec), although we cannot entirely preclude the possibility. The discrepancy between the results seems more likely to be attributable to variability of the *in vitro* preparation's responsiveness to dopamine (Zapata, 1975) or to differences in dose used and the time during which the drug is present in the carotid body. The delayed excitatory effect observed *in vivo* may be mediated by a mechanism which perhaps involves a different type of dopamine receptor, but further studies are required to characterize this response.

Dopamine appears to be involved in modulating chemosensory activity in the cat (Mitchell & McDonald, 1975; Zapata, 1975; Docherty & McQueen, 1978*a*) and it is tempting to speculate that it has a similar role in the rabbit carotid body. However, this remains to be established.

Physiological significance. It is difficult to reconcile the present finding that ACh inhibits rabbit carotid chemosensory activity with the hypothesis that ACh is an *excitatory* transmitter in the chemosensory mechanism (e.g. see reviews by Heymans & Neil, 1958; Eyzaguirre & Zapata, 1968; Torrance, 1968; Biscoe, 1971; Howe & Neil, 1972). The nicotinic action of ACh in exciting sensory nerve endings may be non-specific, that is a direct action on the nerve endings which can be blocked pharmacologically without affecting the response of the endings to physiological stimuli (Brown & Gray, 1948; Gray & Diamond, 1957). It could be that whereas non-specific excitation of chemosensory afferents is observed in the cat and dog, it is not obtained, except transiently following high doses of ACh, in the rabbit. Could the threshold for non-specific excitation by ACh be higher in the rabbit than in other species? Is this related to levels of acetylcholinesterase? One could start speculating as to why inhibition of chemosensory activity occurs in rabbits, but it would be more profitable to study further the properties of the rabbit carotid body and sinus nerve and to compare them with those of the cat and dog (e.g. Verna's (1975) ultrastructural study of the rabbit carotid body).

It may well be that the inhibition evoked by ACh in rabbits is either indirectly mediated or else non-specific and lacking physiological significance, but it seems

worth exploring the possibility that these findings with exogenous ACh in rabbits, free from any masking excitatory effect of the drug, provide a clue regarding the physiological role of ACh in the carotid body. There is evidence for an efferent pathway running in the sinus nerve to the cat carotid body (e.g. see Biscoe & Sampson, 1968) and stimulation of this pathway inhibits carotid chemosensory activity (Neil & O'Regan, 1969; Belmonte & Eyzaguirre, 1974). Several workers have reported that this inhibition can be reduced by atropine (Willshaw, 1975; Goodman, 1975; Sampson, Aminoff, Jaffe & Vidruk, 1976*b*), although opinions differ about the mechanism of the inhibition and the physiological significance of the pathway (McCloskey, 1975). It remains to be established whether the rabbit carotid body receives an efferent innervation via the sinus nerve, but the possibility exists that the inhibitory effect of ACh on chemosensory activity in rabbits might result from an action on receptors involved in an efferent pathway. In such a scheme ACh would be a *modulator* or *regulator* of chemosensory activity rather than an excitatory neurotransmitter.

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EFFECTS OF ACETYLCHOLINE AND SODIUM CYANIDE ON CAT CAROTID BARORECEPTORS

D.S. McQUEEN

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ

1 The effects of intracarotid (i.a.) injections of acetylcholine (ACh) and sodium cyanide (NaCN) on baroreceptor activity recorded from the sinus nerve have been investigated in cats anaesthetized with pentobarbitone.

2 Two types of baroreceptor unit were recorded. The predominant type discharged at least 3 to 4 spikes per pulse wave at normal BP; they are referred to as 'polyspike' units and may have been associated with A fibres. The other type discharged a maximum of 1 to 3 spikes per pulse wave, even at high BP; they are referred to as 'few-spike' units and may have been from C fibres.

3 NaCN had no direct effect on either type of baroreceptor unit, even when injected in high doses (2.04 to 5.1 μmol i.a.) which cause maximal chemoreceptor stimulation, and it is concluded that as far as the cat's carotid baroreceptors and chemoreceptors are concerned, NaCN is a specific chemoreceptor stimulant.

4 ACh had no direct effect on polyspike baroreceptor units unless very high doses (1.83 μmol i.a.) were injected, when there was occasionally a transient slight increase in discharge. This effect appeared to be secondary to muscle contraction caused by ACh since it was not seen when an adequate neuromuscular-blocking dose of gallamine had been administered.

5 ACh stimulated the few-spike type of baroreceptor unit, an effect which was dose-related and lasted for up to 3 s; the threshold dose for baroreceptor stimulation was higher than that needed to excite chemoreceptor units. The increased discharge also occurred during experiments in which gallamine had been administered. Only five of these units were recorded during the investigation, despite an intensive search for them.

6 There was a delayed increase in baroreceptor sensitivity following the administration of ACh in doses (37 to 366 nmol i.a.) which had no immediate direct effect on polyspike baroreceptor discharge. The effect was evidently not secondary to changes in sympathetic nerve activity to the sinus region since it was observed during an experiment in which the ganglioglomerular nerves had been cut. Whether the increased sensitivity resulted from direct or indirect actions of ACh remains to be determined.

7 It is concluded that low doses of ACh or other drugs with nicotinic properties are unlikely to evoke baroreceptor reflexes on intracarotid injection, although they may cause delayed changes in baroreceptor sensitivity. Higher doses of ACh do not directly affect baroreceptor polyspike (A fibre) units, but transient baroreflex changes might result from stimulation of baroreceptor few-spike (C fibre) units. It is most unlikely that NaCN has any direct effect on baroreceptor reflex activity when injected into the carotid artery in doses used to elicit chemoreceptor reflexes.

Introduction

Sodium cyanide (NaCN) is a classical chemoreceptor stimulant (see Heymans & Neil, 1958). However, Fahim, Paintal & Torrance (1972) have found that some aortic chemoreceptors in cats were not excited by NaCN and some gastric stretch receptors were. They conclude 'that NaCN is definitely not a specific chemoreceptor stimulant'. Furthermore, Dontas (1954) claimed that NaCN exerted a stimulant action on the carotid baroreceptors and Paintal (1977) considered that 'it would not be surprising if sodium cyanide also stimulated the carotid baroreceptors with non-medullated fibres'.

These points raise the question of whether investigators who used NaCN during the study of chemoreceptor reflexes (e.g. see Heymans & Neil, 1958; McQueen, 1970) were evoking responses which were the result of mixed baro- and chemoreceptor stimulation. They also cause one to question whether the

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identity of a unit recorded from the sinus nerve can be based, reliably, on its responsiveness to NaCN. It was decided to perform an electrophysiological study of the effects of NaCN on carotid baroreceptors in the cat in order to try and answer these questions. Responses to acetylcholine (ACh) were also investigated because there is some confusion in the literature concerning the effect of this drug on the baroreceptors. Euler, Liljestrand & Zotterman (1941) found that ACh increased chemoreceptor discharge without affecting the 'great pressure spikes' in recordings of sinus nerve activity, and Landgren, Skouby & Zotterman (1953) considered that ACh was not an adequate stimulus for the baroreceptors, although it did alter their sensitivity to other stimuli. Diamond (1955) used an *in vitro* preparation of the carotid sinus and found that ACh stimulated the carotid baroreceptors.

Methods

Experimental animals

Cats of either sex weighing between 2.1 and 3.9 kg (median weight 2.9 kg, $n = 26$ cats) were used. They were anaesthetized with pentobarbitone sodium (42 mg/kg i.p.) supplemented approximately every 1 to 2 h during the experiment by intravenous administration of 10% of the initial dose. For some of the experiments the animals breathed spontaneously, but for the majority they were artificially ventilated with room air and usually paralysed by gallamine triethiodide (3 mg/kg i.v.). End-tidal CO_2 was continuously monitored by an infra-red CO_2 analyser (Med 1A; Grubb Parsons) and the Pa_{CO_2}, Pa_{O_2} and pH of femoral arterial blood samples measured at regular intervals.

General details

Blood pressure was recorded from one femoral artery and the other was cannulated for arterial blood sampling. Rectal temperature was maintained at 38 ± 0.5°C by a heating pad and the bladder was drained regularly.

Drug solutions (0.1 ml) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded and washed in with 0.2 ml modified Locke solution which had been bubbled with 5% CO_2:95% air in a water bath at 37°C. The catheter was introduced into the common carotid artery via the lingual artery and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. Injections were made over a 2 s period.

Recording and analysis of sinus nerve activity

This has been fully described previously (McQueen, 1977; Docherty & McQueen, 1978) and only a brief summary follows. A carotid sinus nerve was dissected free from surrounding tissues, cut centrally, and the electrical activity of single or few unit baroreceptor units recorded from the peripheral nerve with bipolar platinum-iridium electrodes and an a.c. amplifier (Neurolog; Digitimer). Nerve activity was recorded on tape (Tandberg 115; d.c.-1250 Hz) and subsequently analysed with the aid of a computer (PDP-8; Digital Equipment Corporation) in order to provide data concerning discharge frequency (e.g. average discharge in ct/s); histograms were obtained from an x–y plotter (Complot, Houston Instruments).

Identification of baroreceptor units

Baroreceptors were identified by the synchrony between the bursts of nerve activity and the rise in pulse pressure. Occlusion of the common carotid artery caudal to the carotid bifurcation led to a reduction or abolition of the discharge which was immediately restored on removing the occluding artery clip. Probing the sinus region caused increased unit activity, whereas injection of 0.3 ml Locke solution saturated with CO_2 had no effect on the discharge, although it strongly stimulated the chemoreceptors. Individual units were identified from the constant shape and amplitude of the action potential.

Drugs

Drugs were prepared in modified Locke solution (McQueen & Eyzaguirre, 1974) and were: pentobarbitone sodium, gallamine triethiodide (May & Baker); acetylcholine iodide, mol. mass 273; sodium cyanide (BDH), mol. mass 49.

Results

Different types of baroreceptor discharge

The most common baroreceptor discharge, obtained in 30 of the 35 units recorded (i.e. 86%), was of the type illustrated in Figure 1, namely polyspike activity associated with the increase in pulse pressure, the number of spikes per beat being related to the mean BP. There was usually no discharge below 40 to 50 mmHg, but above this threshold baroreceptor discharge increased with increasing pressure until a maximum discharge was attained at about 200 mmHg. At physiological pressures (90 to 150 mmHg) there were always at least 3 spikes per beat, usually 5 to 15, from a single unit.

The other type of activity observed is illustrated in Figure 3. This was encountered in 5 of the 35 (14%) units studied and was characterized by having a maximum of 1 to 3 spikes per beat and a higher threshold

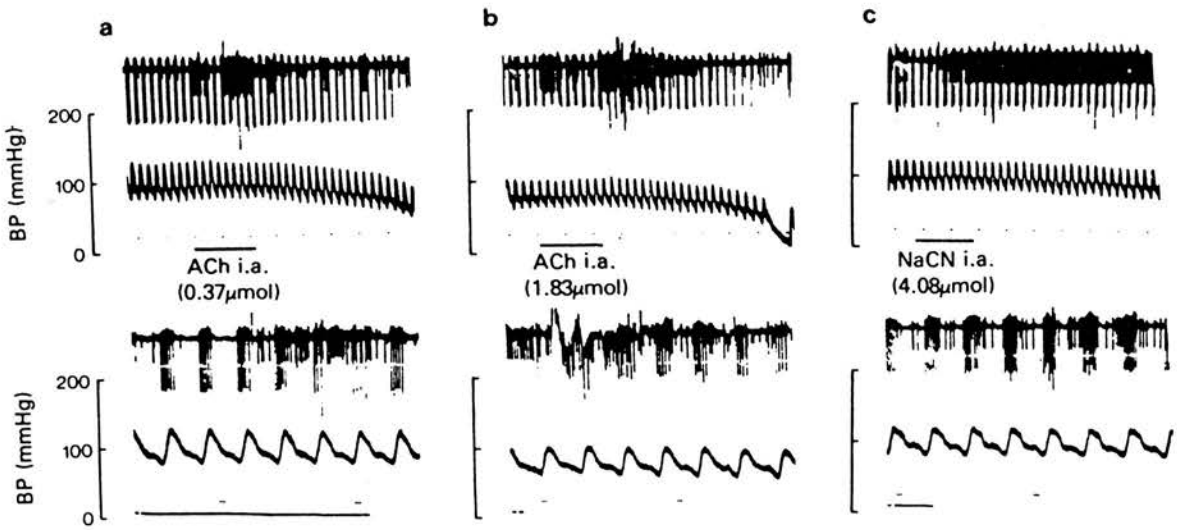

Figure 1 Effects of acetylcholine (ACh) and sodium cyanide (NaCN) on baro- and chemoreceptor activity in a non-paralysed spontaneously breathing cat with intact ganglioglomerular nerves: (a) shows the response to ACh (0.37 μmol i.a.), the lower panel being a faster oscilloscope sweep of the injection period. It can be seen that this dose of ACh markedly increased chemoreceptor discharge (smaller units) without affecting baroreceptor activity (larger unit). When the dose was increased to 1.83 μmol ACh, (b), muscle contraction occurred and the signal was distorted by movement of the nerve on the recording electrodes. A high dose of NaCN (4.08 μmol) (c) caused strong chemoreceptor excitation, but had no effect on baroreceptor activity. Panels show from above downwards: nerve action potentials; femoral BP; 1 s time marker; injection marker.

(mean BP, 70 to 100 mmHg). It was found that the duration of the action potential tended to be longer for the 'few-spike' group (1.5 to 4 ms) than it was for the 'polyspike' group (0.5 to 1.5 ms).

An assumption was made, and will be justified in the Discussion, that polyspikes are associated with fast-conducting (A) fibres in the sinus nerve, whereas the 'few-spike' type of activity is associated with slow-conducting (C) fibres.

Responsiveness of baroreceptor polyspike units (A fibres) to acetylcholine and sodium cyanide

ACh and NaCN were tested on 30 polyspike recordings obtained from 24 cats. Doses injected ranged from 3.7 nmol to 1.83 μmol (i.a.) for ACh and 20 nmol to 5.1 μmol (i.a.) for NaCN. The discharge pattern during the first 5 to 10 s following an injection was compared with that observed following a control injection of the same volume of Locke solution, which occasionally evoked a slight increase in discharge during the injection period. It was evident from early results that neither drug was having much effect on baroreceptor activity, so in subsequent experiments only the higher doses were studied.

Gallamine Experiments were performed on 5 cats which were not paralysed. It was found that higher doses of ACh (≥ 0.18 μmol) caused a transient contraction of the muscles in the neck which often resulted in movement of the nerve on the recording electrodes and distortion or loss of the signal. There was no evidence of baroreceptor stimulation following high doses of ACh, although the situation during the period of muscle contraction was difficult to assess, nor did high doses of NaCN affect the discharge (see Figure 1).

In the other 19 cats, gallamine was administered to prevent muscle movements from affecting discharge. Neither ACh or NaCN in high doses had any stimulant action on the baroreceptor units in 16 of the experiments. However, in 2 cats ACh (1.83 μmol i.a.) did increase discharge during the injection (see Figure 2). This effect coincided with slight muscle contraction in the neck, indicating that there was insufficient gallamine present to prevent the high dose of ACh from contracting muscles and thereby transiently affecting discharge. Again, NaCN was without effect.

Sympathetic innervation of the carotid sinus The ganglioglomerular nerves (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961) were left intact in seven experiments and cut in the remainder. ACh and NaCN were without effect in all the experiments in which the sinus symapthetic innervation was intact. Five of the cats were paralysed with gallamine, two were not.

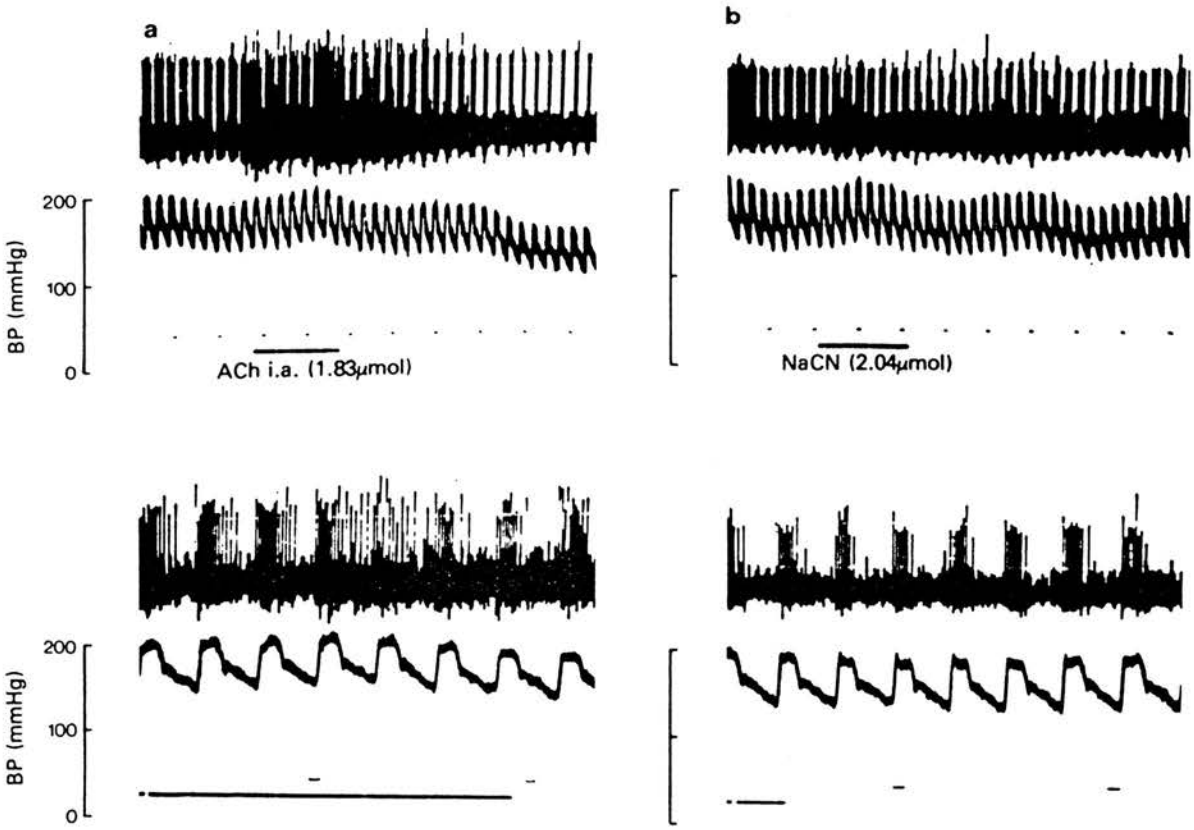

Figure 2 In this experiment the cat was artificially ventilated, paralysed by gallamine and the ganglioglomerular nerves were cut: (a) shows the effect of acetylcholine (ACh, 1.83 μmol i.a.), the lower panel being a faster sweep of the injection period; (b) is the response to sodium cyanide (NaCN, 2.04 μmol). Both drugs stimulated the small chemoreceptor units and ACh, but not NaCN, caused a transient excitation of the baroreceptors, an effect which coincided with muscle contraction in the neck. Record details as for Figure 1.

Responsiveness of baroreceptor few-spike units (C fibres) to acetylcholine and sodium cyanide

ACh and NaCN were tested on 5 few-spike recordings obtained from 5 cats in which the ganglioglomerular nerves had been cut. Two of the animals were paralysed with gallamine, the others were not. Doses of ACh (37 nmol to 0.92 μmol) and NaCN (0.1 to 2.04 μmol) were injected (i.a.) and it was found that whereas higher doses of ACh sometimes increased baroreceptor activity, NaCN was without effect. The biggest response to ACh was obtained from an experiment in which gallamine had been administered (see Figure 3). The lower dose of ACh had only a slight effect on discharge, but the high dose caused an intense discharge, unrelated to pulse pressure, which lasted for 3 s. A high dose of NaCN did not cause any increase in discharge.

Not all these units displayed the same sensitivity to ACh. Thus, the baroreceptor shown in Figure 4a was excited by ACh (0.18 μmol), the recording having been obtained from a gallamine-treated cat, whereas that shown in Figure 4b was unaffected by this dose of ACh. An attempt was made to determine whether the latter unit responded to 0.92 μmol ACh, but unfortunately the dose caused violent muscle contractions and the recording was lost, this being one of the hazards of injecting large amounts of ACh into non-paralysed animals. NaCN (1.02 μmol i.a.) had no effect on either unit.

Sensitisation of baroreceptors

The effect of various doses of ACh (37 nmol to 1.83 μmol) was studied on a single polyspike baroreceptor recorded from an artificially ventilated paralysed ani-

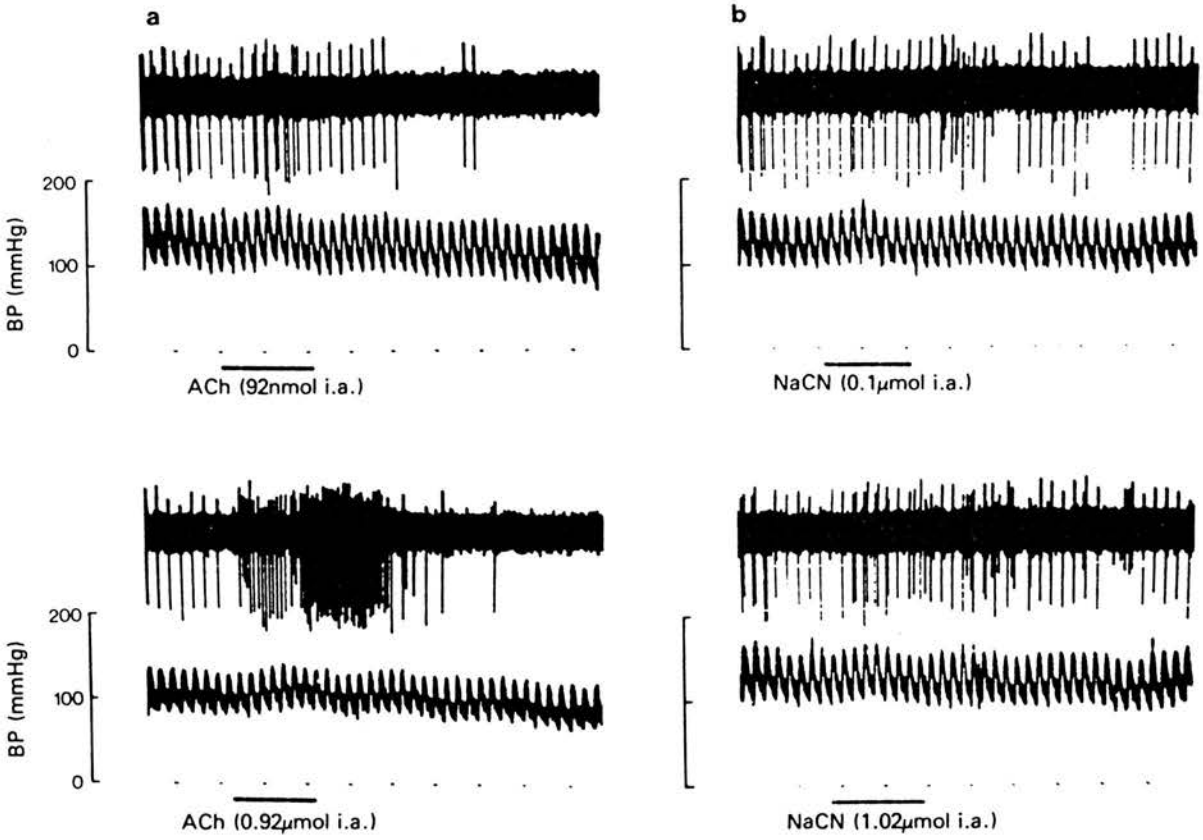

Figure 3 A recording of 'few-spike' baroreceptor activity from a paralysed cat in which the ganglioglomerular nerves were cut: (a) shows the responses to 92 nmol and, below, 0.92 μmol of acetylcholine (ACh). The low dose caused a slightly increased baroreceptor discharge and the high dose caused a marked increase in activity. In contrast, sodium cyanide (NaCN, 0.1 and, below, 1.02 μmol) had no effect on the discharge. Record details as for Figure 1.

mal in which the ganglioglomerular nerves were cut. It was noted that discharge was potentiated during the period following the hypotension evoked by lower doses of ACh, at a time when mean BP and pulse pressure were about the same as in the control period (see Figure 5). Discharge was not increased above control levels following higher doses of ACh, but mean BP remained below control levels during the first 90 s after the injections. Allowing for the lower BP, discharge was, in fact, potentiated. The increased discharge could not be accounted for entirely by the increase in heart rate, although this did make a small contribution.

Discussion

None of the baroreceptor units recorded was stimulated by NaCN, despite the use of high doses which cause maximal chemoreceptor excitation (McQueen, 1977). Dontas (1954) presented no evidence to support his assertion that NaCN (0.1 to 1 mg, i.a.) stimulates baroreceptors in cats and dogs, and Paintal (1977) was merely speculating when he suggested that cyanide might stimulate baroreceptors with non-medullated fibres. The lack of evidence in the literature to support the notion that NaCN stimulates baroreceptors, and the present failure to demonstrate any such effect, makes it reasonable to conclude that low doses of NaCN used to activate the chemoreceptors during the study of carotid chemoreceptor reflexes will not directly affect the baroreceptors.

In contrast to the findings of Fahim *et al.* (1972) on *aortic* chemoreceptors, none of the *carotid* chemoreceptor units obtained by the author from over 250 recordings in 168 cats has failed to respond to NaCN (0.1 μmol i.a.). It appears, therefore, that as far as the cat carotid baroreceptors and chemoreceptors are

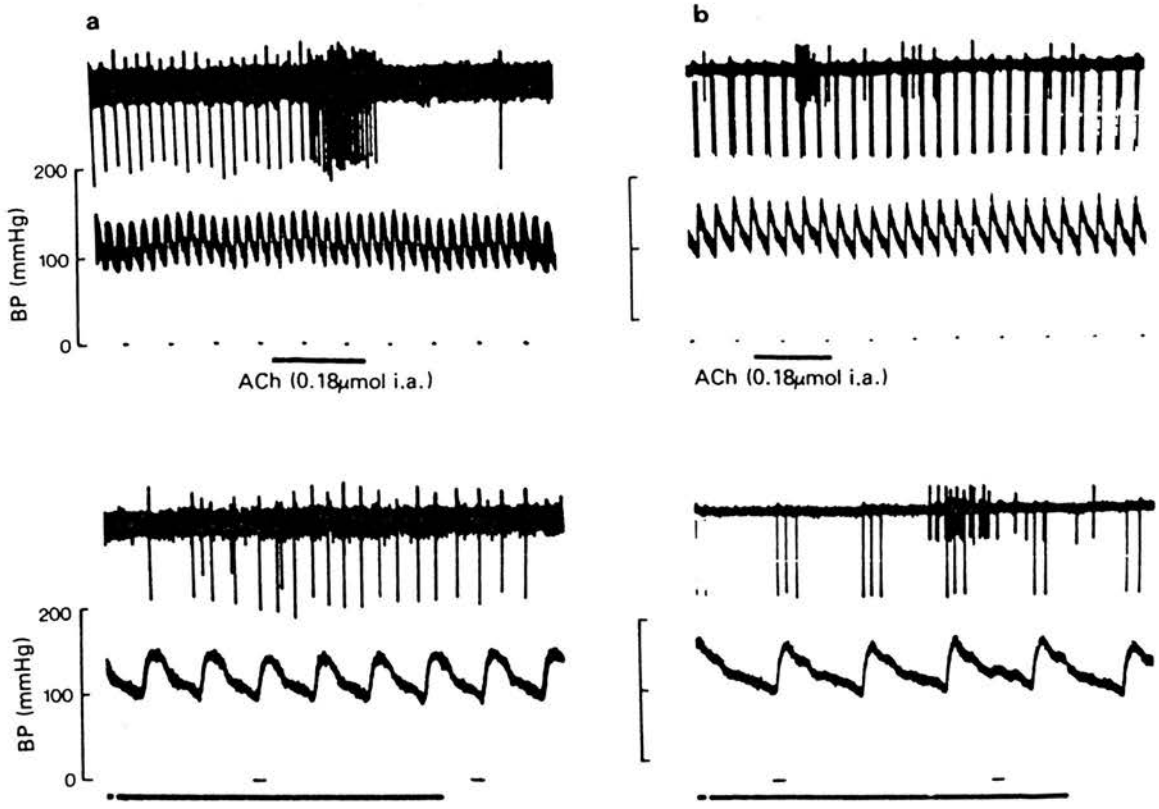

Figure 4 Responses to acetylcholine (ACh, 0.18 μmol) recorded from 'few-spike' recordings of baroreceptors in two different animals. The responses shown in (a) was from a paralysed cat; a fast sweep of the injection period is shown below the slow oscilloscope sweep. Discharge was increased in this experiment, whereas in (b) a recording from an unparalysed cat showed no baroreceptor response to the same dose of ACh, although chemoreceptor units were stimulated. Record details as for Figure 1.

concerned, NaCN is a specific chemoreceptor stimulant. With ACh the situation is more complicated. 'Polyspike' baroreceptor units were only affected by very high doses of ACh, and this effect was evidently secondary to muscle contraction since it was not seen when the neuromuscular blocking drug gallamine was administered in doses which have no effect on the response of the chemoreceptors to ACh (McQueen, 1977).

The 'few-spike' baroreceptor units, in contrast, tended to be excited by ACh, although the doses needed to do this were greater than those needed to stimulate chemoreceptors. The effect was not due to ganglionic stimulation by ACh of the sympathetic supply to the carotid sinus (Kezdi, 1954; Sampson & Mills, 1970; but cf. Floyd & Neil, 1952; Simón, Zamorano, Yajeya & Belmonte, 1976) because it was obtained during experiments in which the ganglio-glomerular nerves had been cut. Neither was it secondary to muscle contraction, since the increase in baroreceptor discharge was observed in paralysed animals.

The only baroreceptor units to respond to ACh (<1.83 μmol i.a.) were of the few-spike type which had long-duration potentials, a feature which could be taken as evidence that they were associated with slow-conducting (C) fibres (Gasser, 1950; Paintal, 1966). It would have been desirable to measure their conduction velocity, but the short length of nerve available makes this technically very difficult (see Paintal, 1971). However, Fidone & Sato (1969) found that: 'baroreceptor C fibres seldom discharge more than 1–2 impulses per pulse wave, whereas baroreceptor A fibres commonly respond with 3–5 impulses or more'. They also found that ACh, in doses greater than those needed to excite chemoreceptor fibres, caused a slight stimulation of baroreceptor C fibres. Thus, the discharge pattern of the few-spike units,

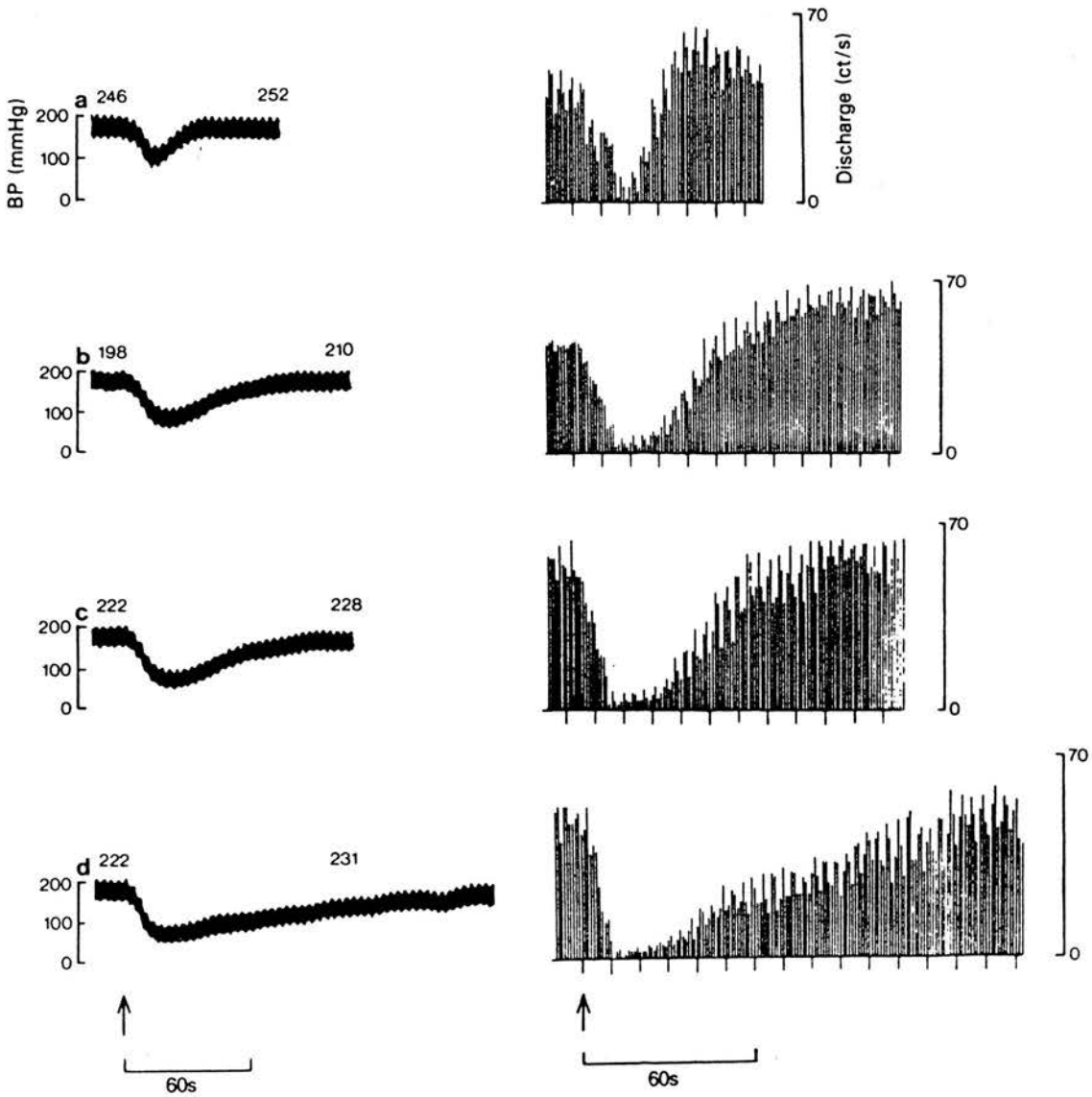

Figure 5 Data obtained from an experiment on an artificially ventilated paralysed cat with the ganglioglomerular nerves cut. Discharge of a single polyspike baroreceptor unit was counted and the computed discharge, plotted by the x–y recorder, is shown on the right of the figure, with the accompanying record of BP on the left. Injections of acetylcholine (ACh) in the following doses were made at the points represented by the arrows: (a) 37 nmol; (b) 0.37 μmol; (c) 0.92 μmol; (d) 1.83 μmol i.a. The average heart rate in the 10 s period immediately preceding the injection was determined, as was the rate during the period 45 to 54 s (a) or 90 to 99 s (b–d) post-injection and the values (beats/min) are given above the BP traces.

together with their responsiveness to ACh (a feature of many non-myelinated fibres, Armett & Ritchie, 1961) strongly suggests that they were baroreceptor C fibres.

The results obtained are not in conflict with the finding of Euler *et al.* (1941) that ACh (5 to 10 μg) has no effect on the great pressure spikes (probably A fibre baroreceptors). Diamond (1955) showed that ACh stimulates the cat carotid baroreceptors *in vitro*, but comparison of his results with those from the

present study is difficult because of the great difference in experimental conditions. He found that small spikes were more affected by ACh than were larger spikes; indeed, some of the latter were unaffected even by very high doses of ACh. This may be taken as indirect evidence that C fibres (small spikes) were more readily affected by ACh than were A fibres (Kirchheim, 1976; but see Iggo, 1958), although it is not clear what proportion of the small spikes were chemoreceptors.

Landgren *et al.* (1953) found that although ACh (10 to 100 μg, i.a.) does not stimulate the baroreceptors, it does increase the receptor sensitivity. Similar findings were made in the present study and further experiments are needed to determine whether this increased sensitivity results from direct or indirect actions of ACh; it is evidently not dependent on intact sympathetic innervation of the sinus because it was observed when the ganglioglomerular nerves had been cut.

In summary, *low doses* of ACh or other drugs with nicotinic properties are unlikely to evoke baroreceptor reflexes on intracarotid injection, although they may cause delayed changes in baroreceptor sensitivity. Higher doses of ACh do not directly affect baroreceptor A fibres, but transient baroreflex changes might result from stimulation of baroreceptor C fibres, although any such changes would probably be masked by the concomitant intense chemoreflex activity evoked by ACh.

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Effects of Dihydro-β-Erythroidine on the Cat Carotid Chemoreceptors

D. S. McQUEEN

From the Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 8JZ, Scotland

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The effects of the nicotinic-blocking drug dihydro-β-erythroidine (DHE) on chemoreceptor responses to physiological and pharmacological stimuli have been examined in pentobarbitone anaesthetized cats in which chemoreceptor discharge was recorded from the peripheral end of a sectioned sinus nerve. High doses of DHE greatly reduced the excitatory action of ACh on the chemoreceptors and caused a reduction in responses to CO_2. The effect of NaCN was not much affected, even though the high doses of DHE can reasonably be expected to have penetrated the carotid body tissues very effectively. Low doses of DHE reduced the excitatory action of ACh but potentiated responses to NaCN and CO_2, findings which are compatible with endogenous ACh exerting an inhibitory influence on the chemoreceptors.

Conflicting evidence and controversy surround the effects of nicotinic antagonists on the responsiveness of carotid chemoreceptors to physiological stimuli and sodium cyanide [e.g. see Landgren, Liljestrand and Zotterman, 1952; Douglas, 1952; Dontas and Nickerson, 1956; Byck, 1961; Joels and Neil, 1962; Anichkov and Belen'kii, 1963; Eyzaguirre and Zapata, 1968a; Sampson, 1971; McQueen, 1977]. Some attempts have been made to explain the reason for this conflict. For example, McQueen [1977] suggested that it results from the subjective interpretation of qualitative pharmacological evidence [see also Dontas and Nickerson, 1956]. Others, however, consider that the conflict arises because some nicotinic blockers, in the doses used, do not penetrate tissues very well, and as a result the local concentration of antagonist at the intrinsic 'receptor site' is insufficient to prevent the action of the endogenous excitatory transmitter presumed to be released by physiological stimuli [e.g. Landgren *et al.*, 1952; Moe, Capo and Peralta, 1948; Eyzaguirre and Zapata, 1968a,c; Nishi and Eyzaguirre, 1971].

Dihydro-β-erythroidine (DHE) penetrates the C.N.S. very rapidly and effectively [see Curtis and Eccles, 1958] and is a good antagonist of the nicotinic action of acetylcholine (ACh) on Renshaw cells [Eccles, Fatt and Koketsu, 1954; Eccles, Eccles and Fatt, 1956; Curtis and Ryall, 1966]. It seemed worth using quantitative neuropharmacological techniques to investigate whether DHE could prevent the effects of physiological and pharmacological stimuli on the cat carotid chemoreceptors *in vivo*, as it is apparently capable of doing *in vitro* [Eyzaguirre and Zapata, 1968b].

In this paper the effects of DHE on chemoreceptor responses to ACh, sodium cyanide (NaCN), carbon dioxide (CO_2) and dopamine are discussed in relation to the physiological significance of ACh in the carotid body.

Methods

Experiments were performed on 7 cats weighing between 2·2 and 4·5 kg, median weight 3·0 kg. Full details of the experimental procedures have been given previously [McQueen, 1977; Docherty and McQueen, 1978] and only a brief summary follows. The animals were anaesthetized with pentobarbitone sodium, (42 mg.kg^{-1} i.p.), artificially ventilated with air and paralysed by gallamine (3 mg.kg^{-1} i.v.). Blood pressure was recorded from a femoral artery, and the lingual artery ipsilateral to the sinus nerve from which recordings were obtained was cannulated, the tip of the catheter being positioned in the common carotid artery about 1·5 cm caudal to the carotid bifurcation. In some experiments a second catheter was introduced into the common carotid artery *via* the superior thyroid artery.

Electrical activity of chemoreceptor units (1–5 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, stored on FM tape (Tandberg 115), passed through a pulse height (window) discriminator, and quantified with the aid of a PDP-8 computer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in modified Locke solution [see McQueen, 1977]. Drug solutions (0·1 ml) were injected into the common carotid artery (I.C.) *via* the lingual catheter and washed in with 0·2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; injections were made over 2 sec. Infusions were made into the common carotid artery *via* the thyroid catheter over a 65 sec period using a Unita pump (Braun) set to deliver 0·5 ml.min^{-1}. The tendency for B.P. to fall following DHE injections was conteracted by slow i.v. injections of dextran 70 (2·5%) glucose (5%) solution (10–60 ml).

Chemoreceptor responses were expressed as changes from pre-injection control level by subtracting the appropriate control value; i.e. as $\Delta\bar{x}$ (change in average discharge during a response of duration t sec) or $\Delta\Sigma x$ (change in total count, i.e. $\Delta\Sigma x = \Sigma x$ (total count in response period) $- \bar{x}$ (control) $\times t$). A 'response' was defined as being from the first substantial change in spontaneous discharge following drug administration until the discharge returned to the pre-injection control level. Responses to chemoreceptor stimulants obtained after administering DHE were expressed as a percentage of the responses evoked by the same doses before DHE was given.

Drugs used were: pentobarbitone sodium, gallamine triethiodide (May and Baker); acetylcholine iodide, sodium cyanide (B.D.H.); dopamine hydrochloride (Koch Light); dihydro-β-erythroidine hydrobromide (Merck, Sharp and Dohme).

Results

Effects of DHE injections on chemoreceptor response to ACh, NaCN, CO_2 and dopamine

Doses of ACh, NaCN, CO_2 and dopamine were injected I.C. in random order with 5 min between successive injections. The sequence was performed before and after injecting DHE (0·1–5 mg.kg^{-1} I.C.). The 0·1 mg.kg^{-1} dose (given i.v.) is effective in greatly reducing Renshaw cell responsiveness to stimulation or ACh in cats [Eccles *et al.*, 1954], but it caused only a 50% reduction in the chemoreceptor excitation evoked by ACh during preliminary experiments in the present series. Consequently, higher doses of DHE (1–5 mg.kg^{-1}) were used in the majority of experiments to ensure a greater antagonism of the ACh response. These doses of DHE caused a fall in B.P., probably as a consequence of ganglionic blockade [Merigan, Leary and Slater, 1955], and this was compensated for by the slow i.v. infusion of dextran-glucose solution.

Figure 1 illustrates the results obtained when DHE 1 mg.kg^{-1} was administered initially and followed by a further dose of 2 mg.kg^{-1} later in the experiment. Responses to ACh were greatly reduced but not abolished by DHE 1 mg.kg^{-1}, whereas those to CO_2 and NaCN were potentiated (dose-response curve shifted to the left). The additional dose of 2 mg.kg^{-1} DHE caused a further reduction in the excitatory response to ACh and an inhibition of the response to CO_2. The potentiation of the NaCN effect which had occurred after 1 mg.kg^{-1} DHE was reversed by 2 mg.kg^{-1}, the responses becoming similar to those in the pre-DHE control (see Fig. 1). After 60 min without drug treatment responses returned towards control levels, although those to ACh were still reduced slightly and the effects of NaCN and CO_2 were slightly potentiated. Thus, the action of DHE (1 mg + 2 mg.kg^{-1} I.C.) lasted for about 60 min.

The difficulty with performing dose-response studies over 50–60 min after administering DHE was that the effect of DHE was gradually wearing off, as could be seen by checking the response to a single dose of ACh at intervals after the administration of DHE. However, the reduction of the ACh effect seemed to be fairly constant for 10–20 min, so it was decided to use only a single submaximal dose of each test substance before and after DHE, which meant that a cycle could be completed within 15–20 min.

The results obtained from single-dose experiments are shown in Fig. 2, which also includes neurograms from an individual experiment. It can be seen that DHE (1 mg.kg^{-1}) substantially reduced the response to ACh, but potentiated the action of CO_2. In terms of the average discharge ($\Delta\bar{x}$), the response to NaCN was decreased by this dose of DHE, whereas the total discharge showed a slight increase. The addition of a further 2 mg.kg^{-1} DHE reduced the responses to CO_2 and NaCN. The greatest reduction in the NaCN response was in $\Delta\bar{x}$, and this arose because the response lasted longer, something which can be clearly seen in the neurograms of Fig. 2. In two experiments a single dose of 5 mg.kg^{-1} DHE virtually abolished the excitatory action of ACh,

reduced that of CO_2, slightly reduced the $\Delta\bar{x}$ response to NaCN, but increased the cyanide-induced $\Delta\Sigma x$ response (see Fig. 2).

Dopamine (5 μg I.C.) inhibited chemoreceptor discharge, as described previously [Docherty and McQueen, 1978]. DHE 1 mg.kg^{-1} reduced the response to 82·3 ± 31 "$_{0}$ ($n = 3$) of the pre-DHE inhibition, a somewhat variable effect as can be seen from the standard error.

Effect of DHE on spontaneous chemoreceptor discharge

Injections of DHE (0·1–5 mg.kg^{-1}) elicited either no change in spontaneous discharge during the 30 sec post-injection period (3 experiments), a slight decrease (1 experiment), or a slight decrease followed by an increase in

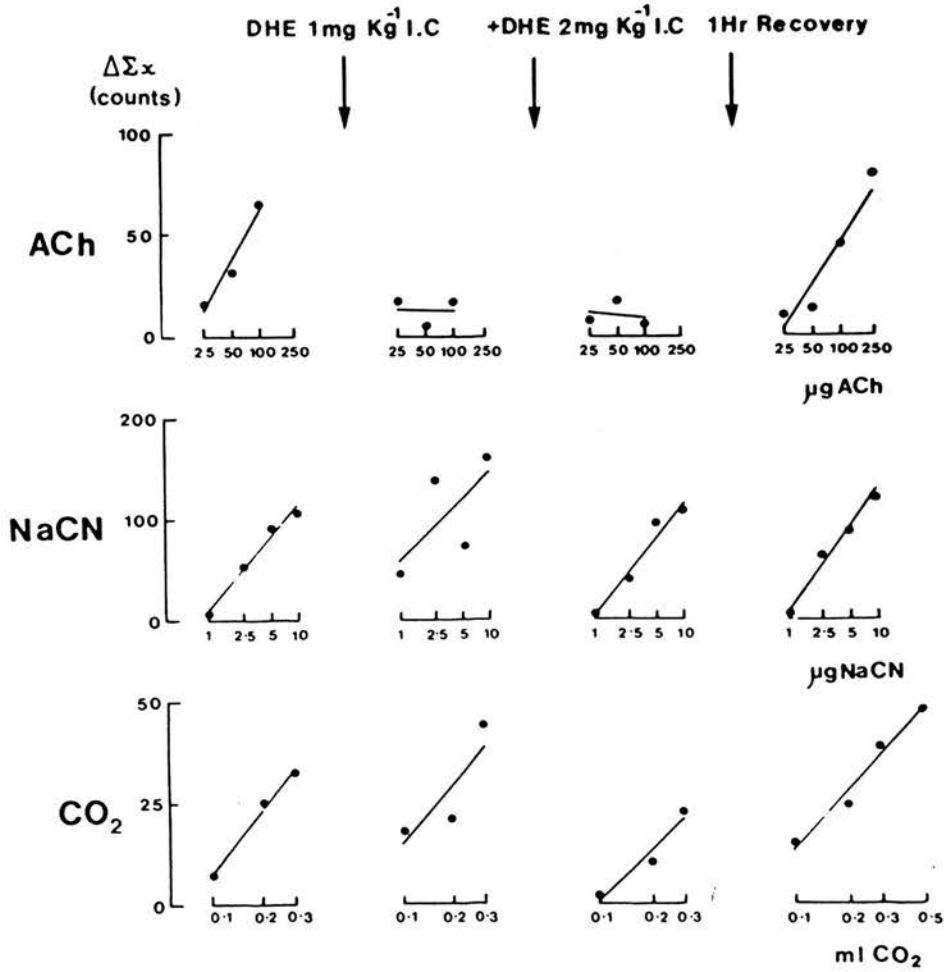

FIG. 1 Effects of injections of DHE on responses of a single chemoreceptor unit to ACh, NaCN and CO_2. The increase in discharge ($\Delta\Sigma x$) has been plotted against $\log_{10}$ dose of stimulant and straight lines fitted to the data by the method of least squares.

discharge (1 experiment). The immediate effects of DHE on spontaneous chemoreceptor discharge were slight and did not appear to be dose-related.

The possibility that DHE had a delayed effect on spontaneous discharge was investigated by comparing the pre-injection spontaneous discharge observed just before injecting single doses of ACh, NaCN, CO_2 and dopamine. Before DHE (1 mg.kg^{-1} I.C.) the average discharge was $1{\cdot}8 \pm 0{\cdot}5$ c.p.s., and this increased to $2{\cdot}4 \pm 0{\cdot}8$ c.p.s. after DHE ($n = 12$ tests in 3 cats), an increase which was not statistically significant ($P > 0{\cdot}05$; Wilcoxon signed rank test for two related samples).

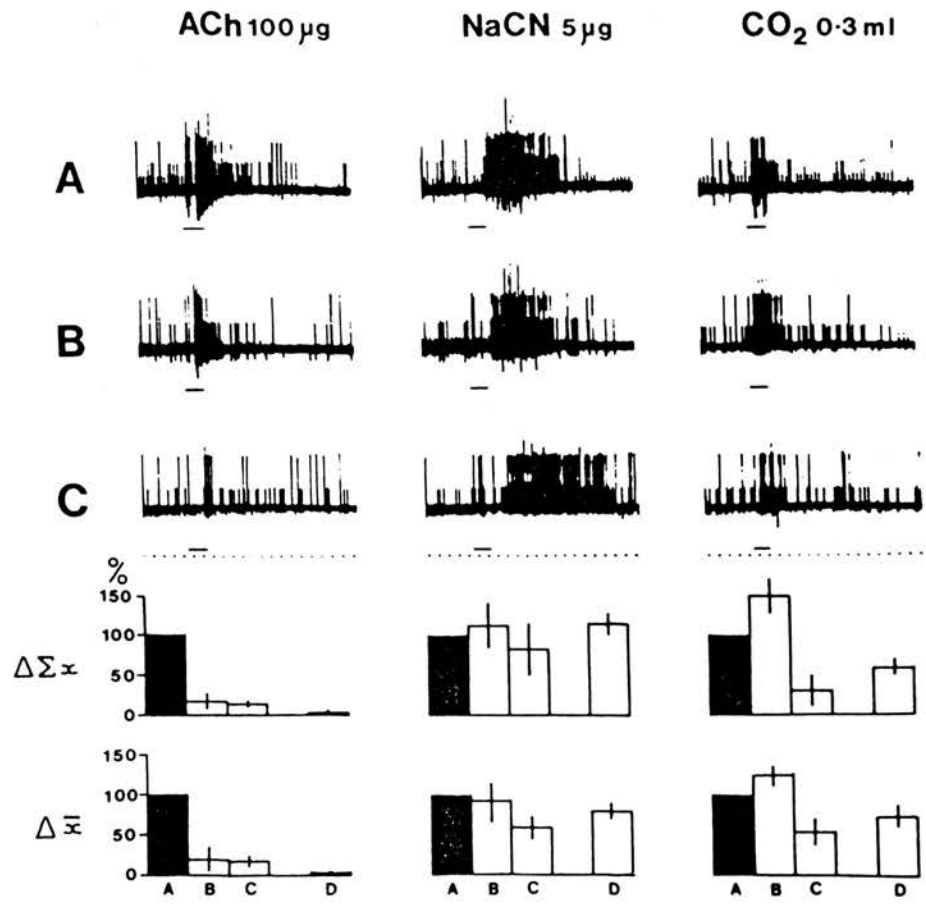

FIG. 2 The upper part of the figure shows neurograms, taken from one experiment, which illustrate chemoreceptor responses to ACh, NaCN and CO_2: *A* before DHE; *B* after 1 mg.kg^{-1} DHE I.C.; *C* after an additional 2 mg.kg^{-1} DHE I.C. Injections were made during the period represented by the short horizontal bar, and the dots are 1 sec time marks.

The lower part is pooled data showing chemoreceptor responses ($\Delta\Sigma x$ and $\Delta\dot{x}$) to ACh 100 µg, NaCN 5 µg and CO_2 0·3 ml expressed as mean percentages ± s.e. of mean of the pre-DHE responses represented by *A* the black rectangles (= 100%). *B* is after 1 mg.kg^{-1} DHE ($n = 3$ cats) and *C* after an additional dose of 2 mg.kg^{-1} DHE. The responses in *D* were obtained following a single dose of 5 mg.kg^{-1} DHE in two experiments.

Effects of DHE infusions on responses to ACh, NaCN, and CO_2

In order to try and obtain a steady-state concentration of DHE in the carotid body, infusions of DHE were made into the common carotid artery *via* the thyroid artery, and test substances injected *via* the lingual artery. Responses were obtained 5 min before starting a 65 sec infusion of DHE, and again 60 sec into the infusion period. Responses to ACh recovered within 20 min of stopping the infusion ($2{\cdot}5\ mg.min^{-1}$), so in two experiments single doses of the test substances were administered in random order with at least 20 min between successive infusions. Results obtained are illustrated in Fig. 3. The low dose of DHE reduced the response to ACh and slightly potentiated the effects of ACh and NaCN. The higher dose ($2{\cdot}5\ mg.min^{-1}$) virtually abolished the excitatory action of ACh and greatly reduced the response to CO_2. The total increase in discharge ($\Delta\Sigma x$) evoked by NaCN was unaltered, but the average ($\Delta\bar{x}$) was reduced (i.e. the same number of impulses occurred, but over a longer time).

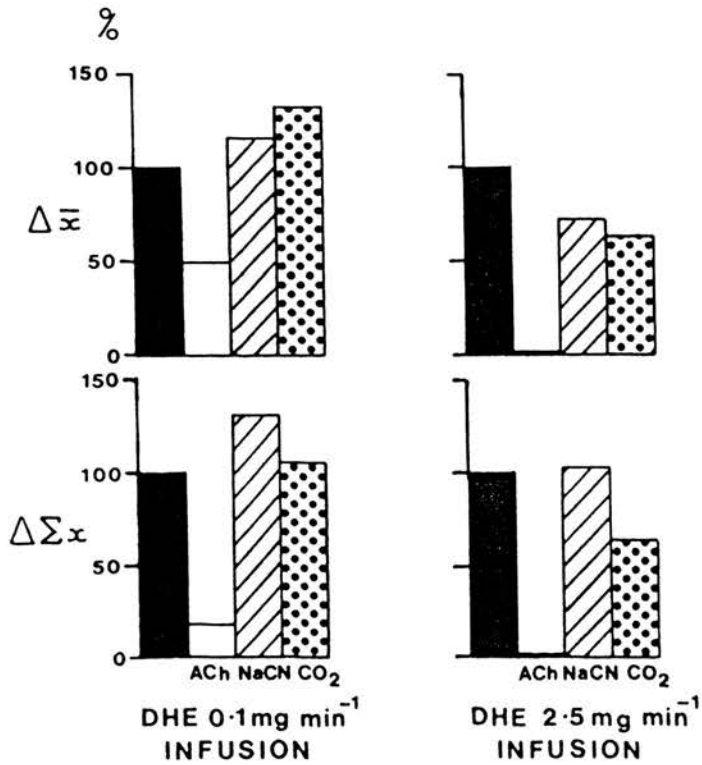

FIG. 3 Effects of infusions of DHE on chemoreceptor responses to ACh, NaCN and CO_2. In one experiment $0{\cdot}1\ mg.min^{-1}$ was infused I.C. for 65 sec in a 4·5 kg cat and the stimulant injected at 60 sec. Responses evoked by the single chemoreceptor unit counted were expressed as a percentage of the response obtained during an infusion of Locke solution (black rectangle = 100%) and are shown as $\Delta\Sigma x$ and $\Delta\bar{x}$.

In a second experiment $2{\cdot}5\ mg.min^{-1}$ was infused I.C. into a cat weighing 3·4 kg and responses obtained from a 3-unit chemoreceptor recording are shown.

The 2·5 mg.min^{-1} infusion caused a slight fall in B.P. which was not compensated.

Discussion

Low doses of DHE greatly reduced the excitatory action of ACh on the carotid chemoreceptors, but did not reduce responses evoked by NaCN or CO_2. Indeed, after injecting low doses of DHE, or during the infusion of low concentrations of DHE, responses to CO_2 and NaCN were *potentiated*. This finding is in accord with previous reports that responses to NaCN can be potentiated by nicotinic-blocking drugs [e.g. Dontas and Nickerson, 1956; Byck, 1961; McQueen, 1977]. These observations are compatible with endogenous ACh exerting an inhibitory effect on the chemoreceptors, a possibility which warrants serious consideration in view of the finding that chemoreceptor inhibition is the predominant action of ACh in rabbits [Docherty and McQueen, 1979].

Higher doses of DHE caused a further reduction in the chemoexcitatory action of ACh, although the inhibition was not as potent or as long-lasting as that caused by mecamylamine [McQueen, 1977], which may mean that the carotid body nicotinic receptor mediating the excitatory action of ACh is more akin to that in ganglia than that at the neuromuscular junction or on Renshaw cells [see Curtis and Ryall, 1966]. The high doses of DHE reduced the excitatory action of CO_2, and this could be interpreted as meaning that ACh is involved as an excitatory neurotransmitter in the process of chemoreception. The fact that the response to NaCN was little affected by DHE, or by mecamylamine [McQueen, 1977; but cf. Nishi and Eyzaguirre, 1971], is not consistent with the 'cholinergic hypothesis', but could be explained as being a consequence of NaCN, unlike physiological stimuli, acting *via* a mechanism not involving an excitatory cholinergic transmitter.

However, the reduction in response to CO_2 was seen only with very high doses of DHE injected close-arterial to the carotid body (i.e. 15 mg single dose), and at these doses DHE may no longer be acting as a selective nicotinic antagonist and could well be affecting other putative neurotransmitters, or their receptors, within the carotid body (e.g. dopamine, noradrenaline, 5-HT, polypeptides). The argument that insufficient blocking drug is reaching the 'synaptic' site at which the postulated excitatory transmitter acts (see Introduction) is not applicable to DHE because the drug penetrates the C.N.S. very well [Curtis and Eccles, 1958], and reasonably be expected to penetrate the carotid body tissues equally effectively. Even if the local concentration of DHE at the 'synaptic' site was insufficient to block completely the responses to NaCN or CO_2 [see Eccles *et al.*, 1954; Curtis and Eccles, 1958] it should have reduced the total discharge evoked. The fact that responses to NaCN and CO_2 were *potentiated* by lower doses of DHE seems more likely to be of physiological interest than the reduction in response to CO_2 seen after massive doses of DHE. However, the possibility that ACh acts as an excitatory transmitter,

although considered very unlikely for the reasons stated, cannot entirely be excluded.

In the present experiments DHE had no immediate or long-term effect on spontaneous chemoreceptor discharge, in contrast to the marked stimulation found *in vitro* by Eyzaguirre and Zapata [1968b], and nor was the reduction in response to ACh as long-lasting as they found. These disparities could result from fundamental differences in the preparations. Changes occur in the *in vitro* carotid body preparation when blood is excluded [Joels and Neil, 1968]—the *in vivo* preparation also has its limitations, such as changes in blood flow which can complicate the interpretation of drug effects.

There is good evidence that ACh is present in the carotid body [Eyzaguirre, Nishi and Fidone, 1972; Fidone, Weintraub and Stavinoha, 1976] and Eyzaguirre's elegant Loewi-type experiments *in vitro* [Eyzaguirre and Zapata, 1968a] suggested that ACh, or a very similar substance, is released during hypoxia. Since the evidence seems to be against ACh being an excitatory transmitter in the carotid body, what is its function there? It could be that during hypoxia ACh is released at more or less the same time as any excitatory transmitter(s), a concept which has been advanced for other tissues [e.g. Jenkins, Marshall and Naysmyth, 1976], or secondary to the increased sensory activity, and acts to modify or inhibit chemoreceptor discharge (as discussed above)—or has some other non-excitatory influence. In the Loewi-type preparation, discharge of the 'downstream' or recipient carotid body may be increased by ACh, released from the hypoxic donor carotid body, acting on the more accessible 'extra-synaptic' nicotinic receptors [Eyzaguirre and Zapata, 1968a] in a non-physiological manner [Brown and Gray, 1948; Gray and Diamond, 1957; McQueen, 1877] and masking any inhibitory action of ACh.

In conclusion, further studies are needed to establish the mechanism whereby ACh exerts its inhibitory action on the carotid chemoreceptors, and to determine whether this effect is of physiological significance.

Acknowledgments

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ALTERED DOPAMINE D_2 RECEPTOR FUNCTION IN THE CAROTID BODY OF RABBITS TREATED CHRONICALLY WITH DOMPERIDONE

D.S. McQueen*, A.K. Mir[+] & S.R. Nahorski[+], Department of Pharmacology, University of Edinburgh, Edinburgh, EH8 9JZ, and [+]Department of Pharmacology & Therapeutics, University of Leicester, Leicester, LE1 7RH.

Previous studies on the rabbit carotid body have provided evidence for a dopamine D_2-receptor which, upon activation, causes depression of chemosensory discharge (Mir et al, 1983). The present study was undertaken to investigate the effect of chronic treatment with the selective dopamine D_2 antagonist domperidone on rabbit carotid body dopamine D_2 receptors, using a combination of biochemical and neuropharmacological techniques.

Rabbits (New Zealand White) were given domperidone orally (2-5 mg/kg/day in drinking water) for 8 weeks and were used for experiments 4-9 days after withdrawal of the drug.

For biochemical studies, carotid bodies were removed from animals under anaesthesia and washed membranes were prepared for D_2 receptor binding assays (Lazareno & Nahorski, 1982). Assays were performed at a single saturating concentration of ^{3}H-domperidone (0.45 nM) and the non-specific binding was defined by 5 µM d-butaclamol. Tissue amine concentrations were also determined using HPLC/EC, as previously described (Mir et al, 1982). Plasma domperidone levels were monitored after oral dosing by modification of the method of Creese & Snyder (1977). Carotid body content (pmoles/carotid body) of noradrenaline 102 ± 28, dopamine 323 ± 57 and 5-hydroxytryptamine 122 ± 38 (n = 5) in domperidone-treated animals were not significantly different from vehicle-treated controls, 85 ± 19, 287 ± 48 and 103 ± 25 (n = 4) respectively, but binding of ^{3}H-domperidone to carotid body membranes was increased by 63% (5.9 ± 1.1 fmoles/carotid body) compared to the control value of 3.6 ± 0.55 fmoles/carotid body ($P < 0.05$). For neuropharmacological experiments, rabbits were anaesthetised with pentobarbitone (40 mg/kg i.v.), artificially ventilated with air and paralysed by gallamine (3 mg/kg i.v.). Electrical activity was recorded from the peripheral end of a sectioned sinus nerve and the depression of chemosensory discharge associated with intra-carotid injection of dopamine was studied (see Docherty & McQueen, 1979). In untreated animals the ID_{50} (injected dose of dopamine causing a 50% reduction in 'spontaneous' discharge averaged over the 5 s post-injection period) was 3.8 ± 0.9 (n = 13) nmoles, whereas in domperidone-treated rabbits the ID_{50} was 0.59 ± 0.26 (n = 3) nmoles ($P < 0.01$). The ID_{50} for the dopamine D_2 receptor agonist LY 141865 (Tsuruta et al, 1981) was 7.3 ± 1.9 (n = 4) nmoles in controls and 2.8 ± 2.1 (n = 3) in the chronically-treated animals.

These results show that following a period of chronic treatment with the dopamine receptor antagonist domperidone, there is an increase in the number of dopamine D_2 receptor binding sites in the carotid body, and this is accompanied by an increase in the chemodepressant effect of injected dopamine. The responsiveness of the chronically-treated animals to physiological stimuli e.g. hypoxia, are being investigated in detail.

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EFFECTS OF SELECTIVE DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS ON CAROTID BODY CHEMORECEPTOR ACTIVITY.

D. S. McQUEEN, Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh, EH8 9JZ, Scotland.

INTRODUCTION

The predominant effect of injecting dopamine into the carotid artery of cats and rabbits in vivo is a depression of carotid chemosensory discharge which can be reduced or abolished by dopamine antagonists such as flupenthixol or haloperidol (e.g. Black et al, 1972; Docherty & McQueen, 1978; 1979).

Various classifications have been suggested for dopamine receptors, but a commonly accepted proposal is that they can be divided into two general categories, D-1 and D-2. Activation of D-1 receptors stimulates adenylate cyclase and leads to increased formation of cyclic AMP, whereas activation of D-2 receptors does not (see Kebabian & Calne, 1979). Dopamine can affect both types of receptor, as can other agonists such as apomorphine and antagonists such as haloperidol. In order to characterise the receptor involved in dopamine-induced depression of chemoreceptor discharge in cats and rabbits, the present experiments were performed using 'selective' dopamine receptor agonists and antagonists. The effects of dopamine receptor antagonists on the responses of chemoreceptors to various physiological and pharmacological stimuli were also investigated.

METHODS

Experiments were performed on seven cats and five rabbits which were anaesthetised with pentobarbitone, paralysed by gallamine (3 mg/kg i.v.) and artificially ventilated with air. Chemoreceptor activity was recorded from the peripheral end of a sectioned carotid sinus nerve (see McQueen (1977) for full details) and the ipsilateral ganglio-glomerular (sympathetic) nerves were cut. Drugs were

injected into the ipsilateral common carotid artery (i.c.). Results were expressed as described previously (McQueen, 1977; Docherty & McQueen, 1978).

RESULTS

The results obtained confirmed that depression of 'spontaneous' chemosensory discharge is the predominant effect of dopamine (0.1-50 ug i.c.) in cats and rabbits. The 'selective' D-2 receptor agonist LY-141865 (Tsurata et al, 1981) proved a very effective depressant of chemoreceptor discharge, being about 50 times more potent and longer-lasting than dopamine in cats, and slightly less potent in rabbits (see Figs. 1 and 2). The D-1 receptor agonist SKF-38393 (Stoof & Kebabian, 1981), in contrast, was very ineffective both in rabbits and cats and was at least 1000 times less potent than LY-141865. Relatively high doses did cause a slight depression of discharge (Figs. 1 and 2).

Experiments with the D-2 receptor antagonists (-) sulpiride and domperidone showed them to be of similar potencies (mw 341 and 397 respectively) in causing dose-related decreases in the chemodepressant responses to dopamine and LY-141865. Threshold effects were obtained with doses as low as 0.1 ug/kg i.c., and higher doses (0.2 mg/kg i.c.) virtually abolished the responses. The effects of domperidone were very long-lasting (> 4 hrs). Both antagonists were effective in cats and rabbits (Fig. 3), and there was little evidence for any appreciable chemoexcitatory action of either dopamine or LY-141865 following substantial reduction of their chemodepressant effects by the antagonists.

Background (spontaneous) chemoreceptor discharge was somewhat variably affected by the D-2 antagonists (Fig. 4), being sometimes increased for long periods following low doses, but in other experiments being unaltered by doses as high as 0.2 mg/kg i.c. In general, there was a tendency for background discharge to increase following domperidone or sulpiride in cats, but there was less effect in rabbits. Responses to stimulants such as hypoxia, cyanide, CO_2 and ACh were also variably affected, particularly in cats. In rabbits responses to hypoxia, cyanide and CO_2 were reduced by domperidone, but the chemo-inhibition associated with ACh was not much affected. Most of the studies were performed after administering 0.01 mg/kg antagonist, but administration of a further 0.2 mg/kg i.c. generally didn't have much additional effect on the evoked responses. The

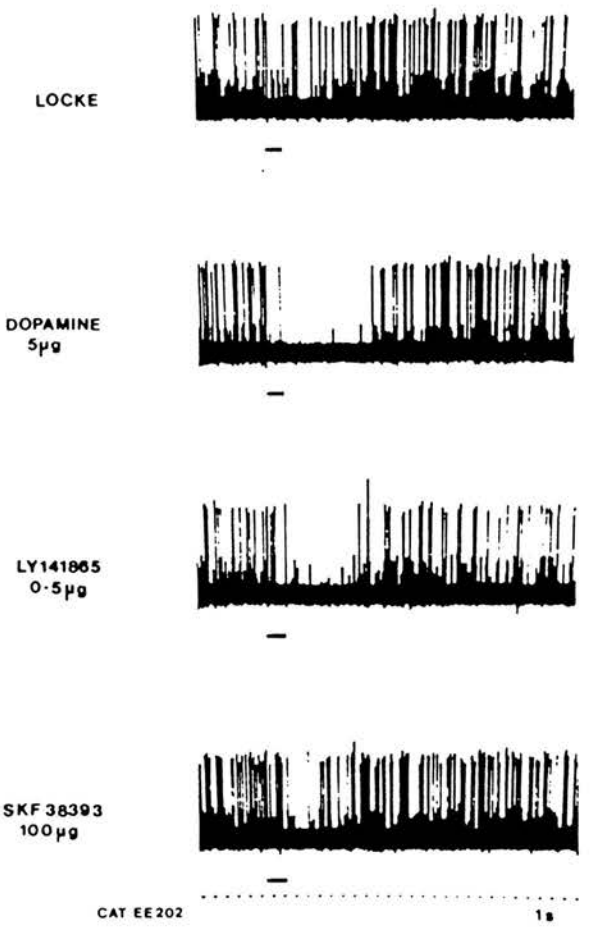

Figure 1. Chemoreceptor discharge recorded from a cat carotid sinus nerve. The neurograms show the effects of injecting (bar) the drug vehicle (Locke solution), dopamine, the D-2 agonist LY-141865, and the D-1 agonist SKF-38393.

Note that LY-141865 is more potent than dopamine, and very much more potent than SKF-38393. The drug vehicle (0.1 ml unbubbled Locke + 0.2 ml wash of Locke bubbled with 5% CO_2 in air) has no appreciable effect on chemoreceptor discharge.

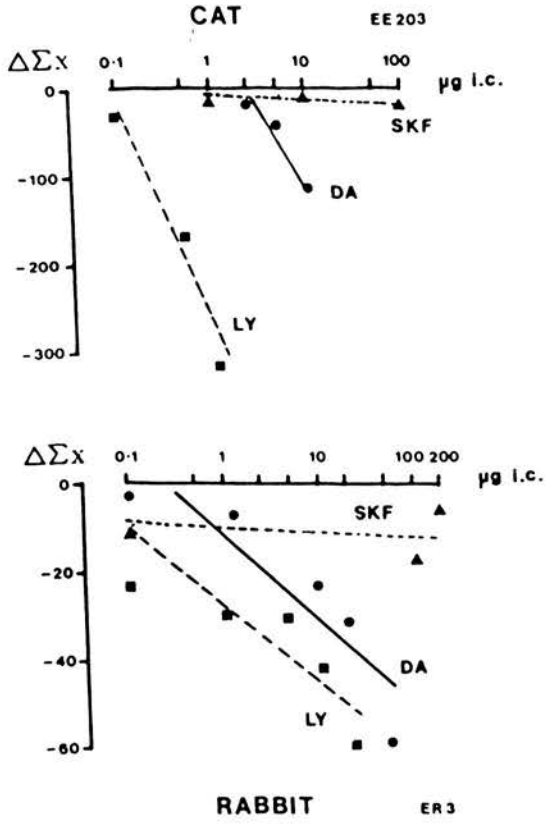

Figure 2. Chemoreceptor dose-response data obtained from a cat (upper part of figure) and a rabbit (lower part). The depression of discharge observed following injection of various dopamine receptor agonists (dopamine, LY-141865, SKF-38393) has been plotted against the dose injected ($\log_{10}$ scale) and lines fitted to the data by eye. The reduction in discharge ($-\Delta\Sigma_X$) is the difference between the number of action potentials counted during the period when the discharge was depressed, and the number anticipated in that period had discharge continued at the pre-injection frequency (see Docherty & McQueen, 1978).

In the cat LY-141865 (mw 292) was about 50 times more potent than dopamine (mw 190), and over 1000 times more potent than SKF-38393 (mw 292) on a molar basis. The corresponding ratios in one rabbit were ≈10 and 2000.

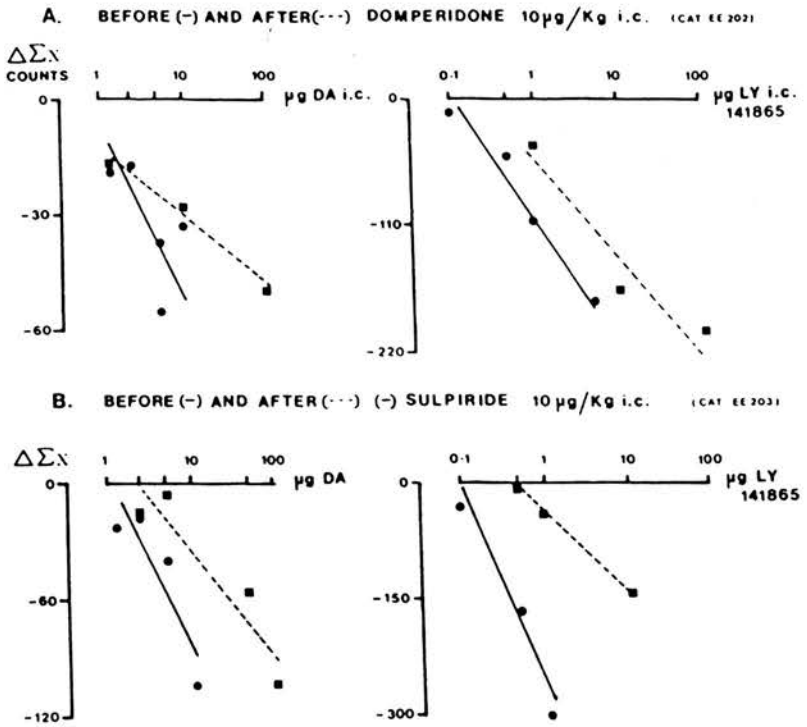

Figure 3. Chemoreceptor dose response data obtained from two cats. The left-hand panels show the chemodepression caused by dopamine before and after injecting a single dose of A domperidone or B (-) sulpiride.

The right-hand panels show the effects of LY-141865 in the same experiments before and after the single dose of A domperidone and B (-) sulpiride. See Fig. 2 for details regarding the method of quantification.

To obtain the same chemodepression after domperidone as before, it was necessary to increase the dose of dopame by a factor of ≈ 6 and that of LY-141865 by 4. The corresponding ratios after (-) sulpiride were 5.5 for dopamine and 14 for LY-141865.

In two rabbits (data not shown) the dose of dopamine had to be increased by an average factor of ≈7 following domperidone (10 µg/kg i.c.), while in another rabbit the ratios were 6 for dopamine and 10 for LY-141865 after (-)sulpiride (10µg/kg i.c).

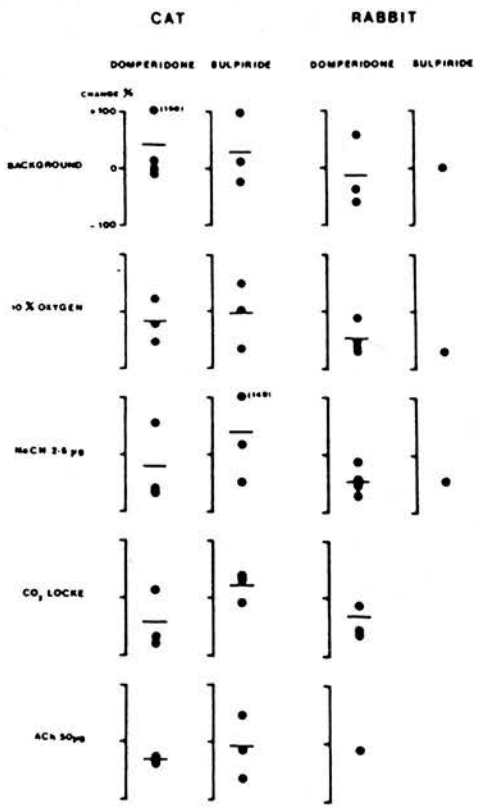

Figure 4. Chemoreceptor activity measured in cats and rabbits after administering domperidone or (different experiments) sulpiride (0.01-0.2 mg/kg i.c.). Results are expressed as a percentage change from control values obtained before the antagonist was given and are shown as the mean (horizontal bar) as well as the individual values, the latter providing an estimate of the variability encountered.

Responses to mid-range doses of NaCN, CO_2-equilibrated Locke solution and ACh were calculated as $\Delta\Sigma x$. For hypoxia, chemoreceptor discharge plateaued about 2 min after switching to ventilating the lungs with $10\%O_2:90\%N_2$, and was then averaged over a 30s period. Background (spontaneous) discharge was averaged from ten separate 30s control periods at different times before and after the antagonist was administered.

depression of chemoreceptor discharge observed following the i.c. injection of either noradrenaline (5 ug) or adrenaline (5 ug) was abolished by (-) sulpiride (0.2 mg/kg i.c.), without affecting the delayed increase in discharge that occurs in cats.

DISCUSSION

If the classification of dopamine receptors into D-1 and D-2 types can be applied validly to the carotid body, then the results obtained with selective agonists and antagonists suggest that the receptor involved in dopamine-induced depression of chemoreceptor discharge in cats and rabbits in vivo has the characteristics of a dopamine D-2 receptor (i.e. which does not stimulate adenylate cyclase). It would appear that adrenaline and noradrenaline can also affect this receptor, directly or indirectly, causing a depression of chemosensory discharge.

The lack of potency associated with the D-1 agonist SKF-38393, coupled with the lack of effect of dopamine after high doses of the D-2 antagonist, suggests that either D-1 receptors are not present in the carotid bodies of cats and rabbits, or else they have no significant effect on chemosensory discharge under the conditions of the present experiments.

Further studies are needed to determine why responses to certain physiological and pharmacological stimuli are variably affected by D-2 antagonists, and these may help to establish the physiological role of the carotid body's D-2 dopamine receptors.

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DISCUSSION

ACKER: Is anything known about the diffusion constants of these drugs. In tumour tissue it has been found that different drugs have different diffusion constants within the tissue. If this applies to your experiments, the actual concentration of the drug at the receptor may be very different for the various compounds.

McQUEEN: I cannot answer that question. I assume that the drugs penetrate readily. The problem is that if you have a compound that appears to be inactive, and I take your point about diffusion, the only way to decide if it really is inactive is to increase the dose. In the in-vivo experiment you then become worried about systemic effects.

NAHORSKI: May I say that it is very unlikely that the selective effects of D1 & D2 antagonists could be explained on the basis of diffusion differences.

EYZAGUIRRE: I think your first slide showed that the depressant effect of dopamine at a dose of 2.5 microgrammes was followed by a rebound excitation. In your last slide, when you applied one of the blockers, the inhibitory effect of dopamine was apparently converted to an excitatory one.

McQUEEN: I find this excitation business very tricky because in general with low doses you never see excitation in the first ten seconds or so. However, in some experiments, you get an increase in the discharge at a later time, whilst in other experiments the discharge returns to pre-injection levels. I have tended to interpret this variability in response at some time after the injection to be due to

changes in the environment of the receptor. Why these changes occur in some experiments and not in others, I have no idea.

EYZAGUIRRE: Do you see this excitation with larger doses?

McQUEEN: With large doses I am concerned about the systemic effects of the compound. Certainly you quite often get an increase in discharge but you always get systemic effects, such as blood pressure changes, at the same time. Hence, once you go more than thirty seconds beyond the primary injection, effects on the chemoreceptors may be indirectly mediated.

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Direct Biochemical and Neuropharmacological Identification of Dopamine D_2-Receptors in the Rabbit Carotid Body

ANIS K. MIR, DANIEL S. McQUEEN[2], DAVID J. PALLOT[1] and STEFAN R. NAHORSKI*

Departments of Pharmacology and Therapeutics, and [1]Anatomy, Medical Sciences Building, University of Leicester, University Road, Leicester LE1 7RH; and [2]Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh, Scotland EH8 9JZ (U.K.)

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Dopamine D_2-receptors were directly identified in receptor binding assays with washed particulate preparations of rabbit carotid body using the selective ligand, [^{3}H]domperidone. High affinity, saturable specific binding of [^{3}H]domperidone was clearly demonstrable and chronic section of the sinus nerve resulted in a 32% decrease in the labelling of the dopamine D_2-sites. Adenylate cyclase activity was also detected in rabbit carotid body homogenates and although this enzyme was stimulated 4-fold by 10 mM sodium fluoride, neither dopamine nor isoprenaline significantly altered basal activity. On the other hand, in the intact carotid body incubated in vitro, 10^{-5} M isoprenaline increased the basal cyclic AMP content 6-fold, though dopamine was again ineffective. The effect of various selective dopamine receptor antagonists and agonists was also studied on chemoreceptor afferent discharge. The results confirm that depression of 'spontaneous' chemosensory discharge is the predominant effect of dopamine (0.01–100 μg) in rabbits. The 'selective' D_2-agonist, LY 141865, proved very effective (ID_{50} 3.3 nmol) and was equipotent with dopamine (ID_{50} 4.2), whereas, the D_1-agonist, SK & F 38393, was very ineffective (ID_{50} 150). The D_2-antagonists domperidone and (—)-sulpiride produced a dose-related decrease in the chemodepressant responses to dopamine and LY 141865. However, there was no evidence for any appreciable excitatory action of either of these agonists after blockade of their chemo-depressant effects. The D_2-antagonists variably affected the spontaneous activity, there being an increase in discharge on average, whereas responses to hypoxia, cyanide and CO_2 were reduced. The present results from biochemical and neuropharmacological studies, provide strong evidence for the presence of functional dopamine D_2-receptors in the rabbit carotid body, and suggest that the receptor involved in dopamine-induced depression of chemosensory discharge is of D_2-type.

INTRODUCTION

The chemoreceptors of the carotid body are sensory receptors which initiate respiratory and cardiovascular reflexes in response to hypoxia, hypercapnia or acidosis[2,15]. There is no agreement about the identity of the primary receptor element (cells or nerve terminals) or what mechanisms are responsible for the onset of sensory discharge in the afferent nerve fibre (see ref. 10). Catecholamines, particularly noradrenaline and dopamine, are present in the Type I cells of various mammalian carotid bodies[26] and changes in the output of these amines during hypoxia[11,14,24] may modulate chemosensory discharge[25,31,38], but their precise function remains to be established.

Studies on the effects of exogeneous dopamine on carotid chemosensory activity have shown that it commonly depresses the chemoreceptors, but can also excite them, or cause biphasic changes in discharge[10]. The suggestion has been made that there are species differences in the response of carotid chemoreceptors to dopamine[4], but since investigators have used different experimental preparations and doses of dopamine, the results obtained are not really directly comparable, regardless of species. Experiments with neuroleptic drugs such as spiroperidol, haloperidol and α-flupenthixol[8,9,38] have shown that these drugs reduce or block the chemodepressant effects of dopamine, but the excitatory effect of dopamine on the chemoreceptors, when obtained, is

* To whom correspondence should be addressed.

generally unaffected. However, although these agents are known to be dopamine antagonists, their specificity towards dopamine receptors is relatively small[32] both in central and peripheral tissues. One interpretation of the available evidence is that dopamine has dual actions and can exert opposing effects on the carotid body chemoreceptors, possibly by acting on more than one type of receptor.

Various classifications have been suggested for multiple dopamine receptors[6,34]. Probably the most widely discussed proposal, however, is that there are two types of dopamine receptors, designated D_1 and D_2[18]. Dopamine D_1-receptors are positively coupled to the enzyme, adenylate cyclase, whereas the dopamine D_2-receptor is either not coupled to the enzyme or is negatively coupled in some tissues[6].

In the present study we describe experiments which were designed to characterize the dopamine receptors of the rabbit carotid body by using a combination of radioligand binding, biochemical and neuropharmacological techniques. The selective dopamine D_2-receptor ligand, [^{3}H]domperidone[1,20] was used to demonstrate the presence and localisation of dopamine D_2-receptors in the carotid body. Adenylate cyclase activity was measured in carotid body homogenates in order to investigate whether the dopamine receptors in this organ are coupled to this enzyme. In addition, we also studied the receptor involved in the depression or inhibition of chemosensory discharge evoked by dopamine in vivo by determining the effects of 'selective' dopamine receptor agonists (LY 141865, D_2[37]; SK & F 38393, D_1[35,36]) and antagonists (domperidone[19], (—)-sulpiride[18,17], D_2) and measuring the response of the chemoreceptors to various physiological and pharmacological stimuli. A preliminary abstract of part of this work has appeared elsewhere[23,27].

MATERIALS AND METHODS

Experiments were performed on male New Zealand white rabbits (2.0–4.5 kg), given food and water ad libitum and kept under circadian illumination.

Surgical procedures

The dissection of the carotid bodies and the procedure for denervation were as previously described[26], and performed in rabbits which were anaesthetized with a mixture of ketamine (22 mg·kg^{-1}) and xylazine (3 mg·kg^{-1}) given intravenously. For electrophysiological recordings the rabbits were anaesthetized with pentobarbitone sodium (35 mg·kg^{-1} i.v. supplemented by administration of 10% of the initial dose every 1–2 h). The trachea was cannulated and the animal artificially ventilated with air and paralyzed by gallamine triethiodide (3 mg·kg^{-1} i.v.). For full details of the experimental techniques see Docherty and McQueen[9].

Recording of chemoreceptor afferent nerve discharge

The carotid sinus nerve on one side was cut centrally, desheathed, and dissected into fine filaments. Electrical activity from chemosensory fibres in the filaments was recorded using bipolar platinum/iridium electrodes and an amplifier system, as previously described and provides an index of activity within the carotid body sensory complex[9,22]. The ipsilateral ganglioglomerular (sympathetic) nerves were cut, and a fine catheter inserted via the lingual artery into the common carotid artery, with its tip positioned about 1 cm caudal to the carotid bifurcation, for close-arterial injection of drugs (0.1 ml washed in with 0.2 ml Locke solution which had been equilibrated with 5% CO_2–95% air at 37 °C over 1–2 s period, with a 5 min interval between doses) to the carotid body. Nerve activity was recorded on magnetic tape and subsequently processed using a pulse height (window) discriminator (WPI 120) to select chemoreceptor potentials (generally 1–3) which were counted every 0.1 s over a period of from 60 to 480 s by a micro-computer (Commodore 3032), which also calculated the mean discharge frequency ($\bar{x}$ cps) and the integrated response ($\Sigma\chi$ counts) of the recorded unit(s) to a drug or procedure.

Radioligand binding

Binding methods were similar to those described previously for rat corpus striatal tissue[20]. Carotid bodies (approx. 1 mg wet weight) were homogenized in 100 vol. ice-cold buffer (Tris-HCl, 50 mM, pH 7.6) with a Polytron homogenizer (setting 6, 15 s) and centrifuged at 48,000 *g* for 15 min at 4 °C in a Sorvall RC5 centrifuge. The pellet was resuspended and the membranes were washed 3 times. The amount of residual catecholamines were assayed in aliquots of the membrane preparation using HPLC

and electrochemical detection as described previously[26]. Membranes (100–200 μg protein) equivalent to two carotid bodies in 2 ml Tris-HCl buffer (50 mM, pH 7.6) were incubated to equilibrium in polypropylene tubes for 30 min at 22 °C for [^{3}H]domperidone and 20 min at 37 °C for [^{3}H]spiperone, collected by rapid filtration through glass-fibre (Whatman GF/C) filters, washed rapidly with 15 ml buffer, suspended in 3.5 ml Fisofluor 1, and the radioactivity was measured in a liquid scintillation counter (Packard) at about 40% efficiency. BSA (0.01%) was used in radioligand stock solutions to reduce the binding of [^{3}H]domperidone to plastic tubes. Specific binding of [^{3}H]domperidone was defined as binding displaced by 5 μM D-butaclamol and that of [^{3}H]spiperone defined by either 50 μM (±)-sulpiride or 5 μM D-butaclamol[20].

Adenylate cyclase activity and cyclic AMP assays

For the measurement of adenylate cyclase activity, tissue was gently homogenized (teflon–glass; 1 carotid body/100 μl) in Tris-maleate buffer (2 mM, pH 7.4; EGTA 2 mM). 100 μl of the homogenate was added to 150 μl of Tris-maleate buffer (pH 7.4 containing $MgSO_4$ 4 mM; EGTA 0.2 mM; IBMX 1 mM) and 50 μl of stimulating drug giving a final concentration of 200 μM dopamine or 10 μM isoprenaline, in the presence of 100 μM GTP. Following a preincubation period of 10 min on ice, the tubes were incubated for 1 min at 30 °C. ATP (50 μl) was added to give a final concentration of 1 mM and the tubes were further incubated for 10 min at 30 °C. Blanks were prepared by boiling immediately after ATP addition. Cyclic AMP generation was also monitored in intact carotid bodies. These were preincubated for 45 min in Krebs–Ringer–bicarbonate buffer at 37 °C, gassed with 95% O_2–5% CO_2. Subsequently, they were transferred to fresh buffer containing the catecholamines, IBMX (1 mM) and 0.2 mM sodium metabisulphite, and incubated for 15 min at 37 °C. In both assays incubations were terminated by transferring to a boiling water bath for 3 min. Homogenates were centrifuged at 10,000 *g* for 15 min and cyclic AMP assayed in 100 μl aliquots of the supernatant using a protein binding saturation method[5].

Drugs

[^{3}H]Spiperone (25.7 Ci/mmol) and [^{3}H]domperidone (59.9 Ci/mmol) were obtained from New England Nuclear and [5′,8-^{3}H]cyclic AMP (45 Ci/mmol) from Radiochemical Centre, Amersham, U.K. Cyclic AMP (free acid), guanosine-5-triphosphate and adenosine-5-triphosphate (Boehringer-Mannheim, Sussex, U.K.). Dopamine hydrochloride, (—)-isoprenaline D-bitartrate, 3-isobutyl-1-methylxanthine (IBMX) and bovine serum albumin (Sigma Chemicals, Dorset, U.K.). The following were generously donated: domperidone (by Janssen Pharmaceuticals, Beerse, Belgium), D-butaclamol (Ayerst, Hants, U.K.), (±)-sulpiride (Delagrange, Paris, France), (—)-sulpiride (Ravezza Pharmaceuticals, Milan), LY 141865 (Eli Lilly, Hants, U.K.), SK & F 38393 (Smith Kline & French, Herts, U.K.).

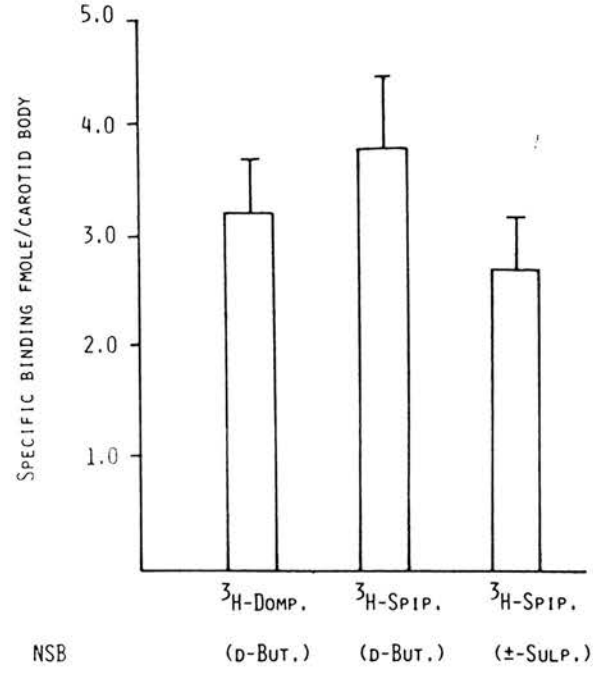

Fig. 1. Membrane preparations of rabbit carotid body were incubated with saturating concentrations of [^{3}H]spiperone (1.0 nM) and [^{3}H]domperidone (0.4 nM). Specific [^{3}H]spiperone binding was measured as the difference between total binding and binding in the presence of either 5 μM D-butaclamol or 50 μM (±)-sulpiride. Incubations were performed at 37 °C for 20 min. Specific [^{3}H]domperidone binding was defined by 5 μM (+)-D-butaclamol and incubations performed at 22 °C for 30 min. Results are expressed as fmol [^{3}H]spiperone and [^{3}H]domperidone bound (carotid body ± S.E.M., n = 3).

RESULTS

[^{3}H]Spiperone and [^{3}H]domperidone binding assays

In initial attempts to directly label putative dop-

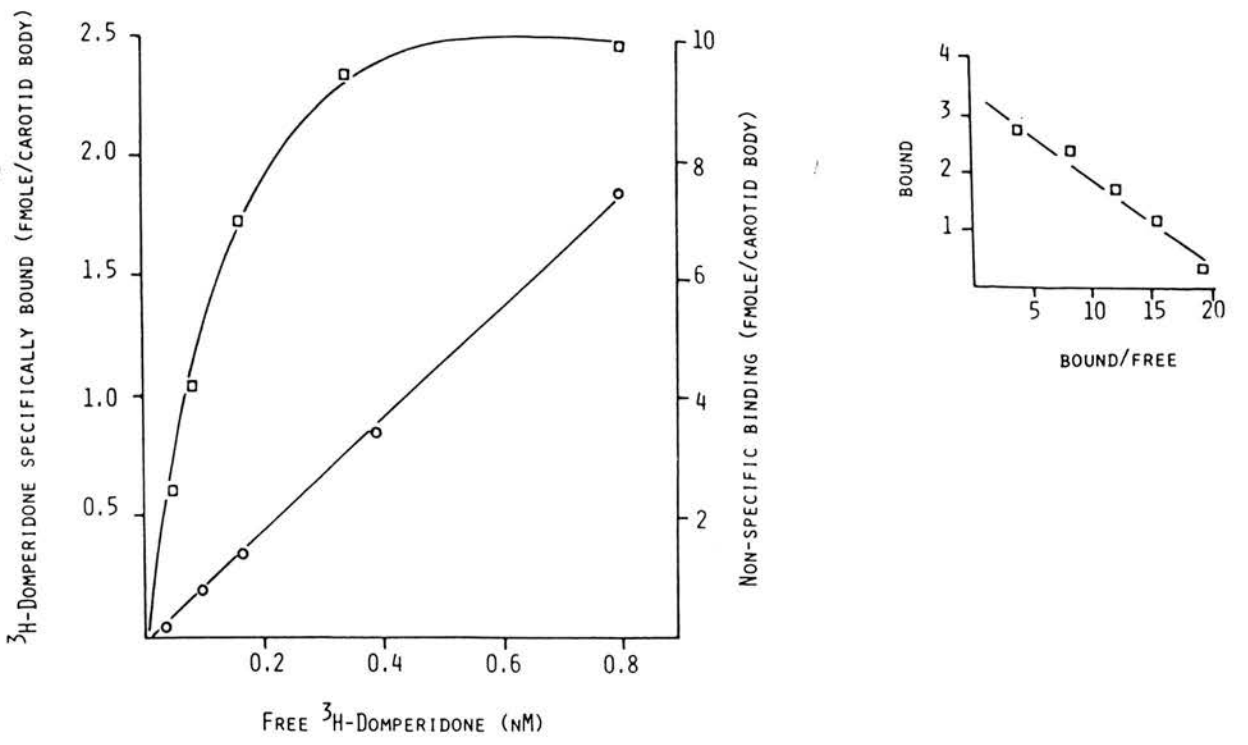

Fig. 2. Increasing concentrations of [^{3}H]domperidone were incubated with rabbit carotid body membrane preparations. Specific binding (□) was measured as the difference between total binding and non-specific binding (○) in the presence of 5 μM (+)-butaclamol. Incubations were carried out at 22 °C for 30 min. The data shown are representative curves for experiments performed in triplicate.

amine receptors in rabbit carotid body-washed particulate membrane preparations, we compared two ligands, [^{3}H]spiperone and [^{3}H]domperidone. The specific binding of these ligands, quantified as the difference between total binding and binding in the presence of 5 μM D-butaclamol, was very similar (Fig. 1). However, in view of experience with these ligands in the central nervous system[16,20] and the indication that specific binding of [^{3}H]spiperone defined by the D_2 antagonist, sulpiride, was lower than that displaced by D-butaclamol, it was decided that the more specific D_2 ligand, [^{3}H]domperidone, would be used in all further studies.

Carotid body membranes were incubated with 5 concentrations (0.02–0.8 nM) of [^{3}H]domperidone for 45 min at 22 °C. Results showed high affinity binding, saturable over the tested concentration range and a specific-to-total binding ratio of about 50% at 0.2 nM [^{3}H]domperidone (Fig. 2). Scatchard analysis of specific binding gave a value for the equilibrium dissociation constant (K_D) of the 0.18 ± 0.05 nM (n = 3) and binding site maxima (B_{max}) 3.2 ± 0.3 fmol/carotid body. Hill analysis of the saturation data gave a Hill coefficient (n_H) of 1.1. The K_D value for [^{3}H]domperidone in the carotid body is very close to that reported for this ligand in the rat striatum[20].

Effect of chronic denervation

In order to obtain information concerning the localization of the specific binding sites, we have examined the effect of chronic denervation on [^{3}H]domperidone binding. Membrane preparations of the normal and denervated carotid bodies were incubated with a single saturating concentration (0.4 nM) of the ligand as described in the text. Chronic denervation of the carotid sinus nerve resulted in a 32% decrease in specific [^{3}H]domperidone binding to denervated carotid body membranes when compared to sham-operated carotid bodies (Fig. 3).

Assay of adenylate cyclase and cyclic AMP in vitro

Homogenates of rabbit carotid body contained a basal adenylate cyclase activity of 0.26 ± 0.08 pmol/

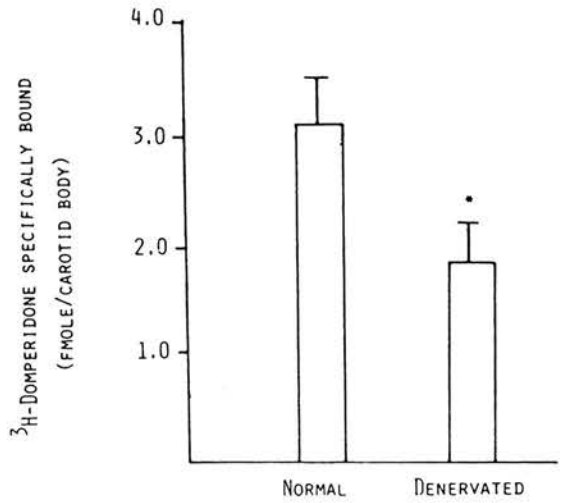

Fig. 3. In a group of rabbits the carotid sinus nerve was unilaterally transected under anaesthesia, 14 days prior to the binding assay. The carotid body from the intact side was used as a control. Membrane preparations of the normal and denervated carotid bodies were incubated with saturating concentrations of [^{3}H]domperidone (0.4 nM) and non-specific binding defined by 5 μM D-butaclamol. Values are expressed as fmol specific [^{3}H]domperidone bound/carotid body ± S.E.M. (n = 3). Asterisk indicates statistical significance compared to the intact carotid body ($P < 0.05$).

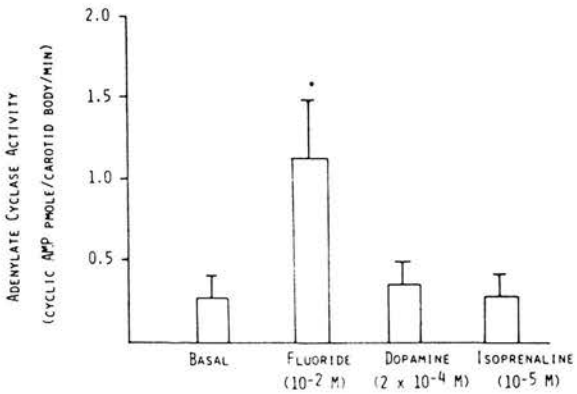

Fig. 4. Homogenates of the rabbit carotid body were incubated in vitro with NaF (10^{-2} M), dopamine (200 μM) and (—)-isoprenaline (10 μM) at 30 °C for 10 min, as described in the text. Control incubations were performed in the absence of NaF and test drugs. Values are expressed as mean with S.E.M. given as bar (n = 4). Asterisk indicates statistical significance as compared to control ($P < 0.05$).

min/carotid body, which was stimulated 4-fold by 10 mM sodium fluoride (Fig. 4). However, concentrations up to 200 μM dopamine and 10 μM isoprenaline did not produce any significant increase in the basal activity. Since dopamine and isoprenaline failed to stimulate adenylate cyclase activity in ho-

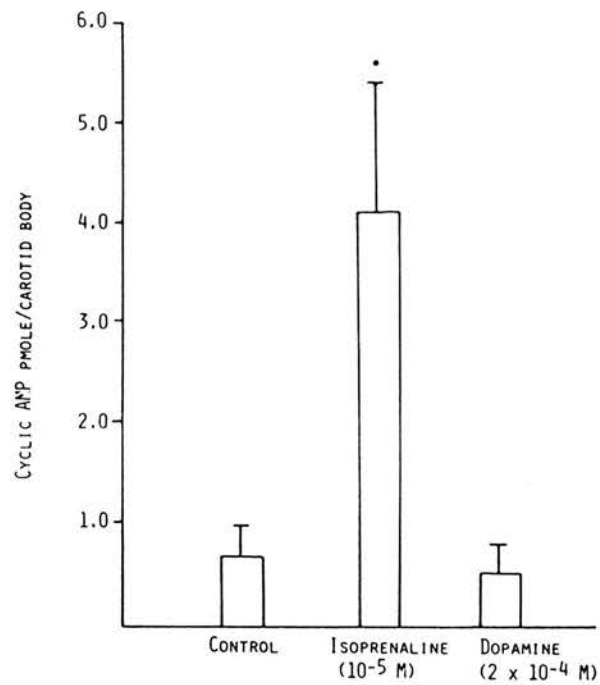

Fig. 5. Intact carotid bodies were incubated in vitro in the presence and absence of (—)-isoprenaline (10^{-5} M) and dopamine (2×10^{-4} M) in Krebs bicarbonate buffer with IBMX (1 mM) for 15 min at 37 °C, as described in the text. Values are expressed as mean with S.E.M. given as bars (n = 4). Asterisk indicates statistical significance as compared to control ($P < 0.01$).

mogenates, it remained possible that this enzyme uncouples from receptors following homogenization. Therefore the ability of dopamine and isoprenaline to stimulate cyclic AMP production, was examined in intact carotid bodies in vitro. Incubation of intact rabbit carotid bodies with 10 μM isoprenaline in vitro for 15 min at 37 °C resulted in a 6-fold increase in cyclic AMP content as compared to the controls (Fig. 5), whereas concentrations up to 200 μM dopamine did not produce any significant change as compared to the control cyclic AMP content.

Neuropharmacological data

Results were obtained from experiments on 7 rabbits. Intra-carotid injection of dopamine (0.01–100 μg) invariably caused a dose-related depression of chemoreceptor discharge which commenced within 1 s of the injection and lasted for 3–60 s (see Fig. 6). There was no evidence for any consistent increase in chemoreceptor activity either immediately on injecting dopamine or in the period following dopamine-induced depression of dis-

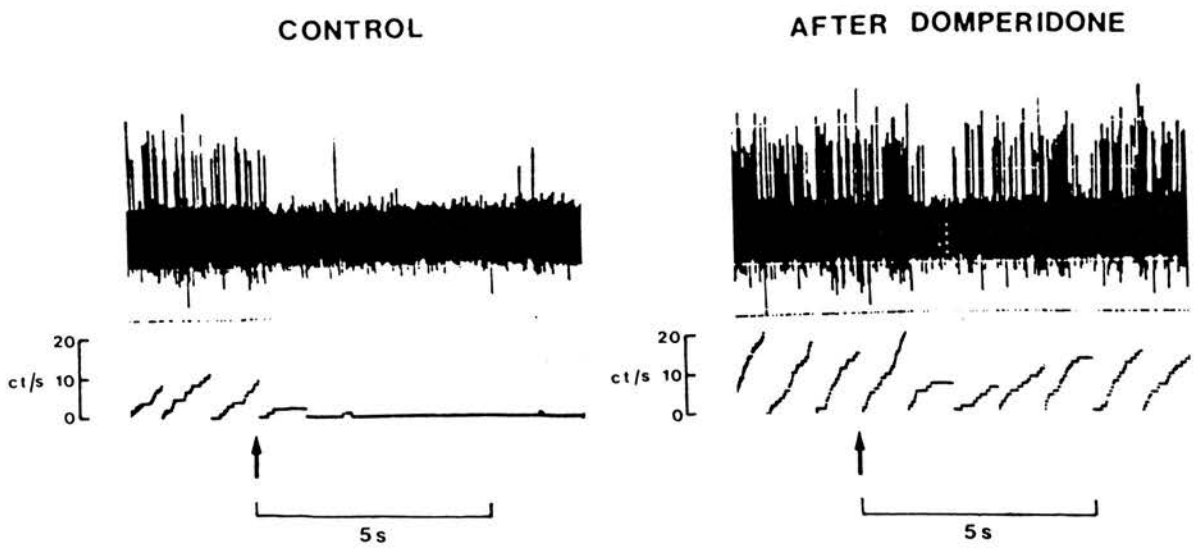

Fig. 6. Neurograms showing chemoreceptor activity recorded from the carotid sinus nerve of a rabbit and illustrating the effect of injecting (arrow) dopamine (5 μg) before and after the antagonist drug domperidone (10 $\mu g \cdot kg^{-1}$ i.c.). The larger action potentials were counted (output pulses from the window discriminator are shown beneath the neurograms) and the ramped trace is the chemoreceptor discharge in cps.

charge, although a delayed increase in discharge was sometimes obtained 15–60 s after an injection.

The changes in chemoreceptor discharge observed following i.c. injections of dopamine, LY 141865 and SK & F 38393 were examined in 3 rabbits and the results evaluated by plotting the overall decrease in discharge ($-\Delta\Sigma\chi$) against the dose of drug injected. Dopamine hydrochloride (mol. mass 190) and LY 141865 (mol. mass 272) were approximately equipotent in depressing discharge, and although SK & F 38393 (mol. mass 272) caused some chemodepression, it was much less effective than the other two agonists (see Fig. 7).

Expressing the results in terms of $-\Delta\Sigma\chi$ means that a maximum response cannot be established since higher doses of the drugs cause longer-lasting effects, up to 2–3 min in the case of LY 141865. This also makes it difficult to evaluate how much of the inhibitory response is a primary effect on the carotid chemoreceptor and how much is secondary to other effects (e.g. cardiovascular) of the injected drug. To overcome the problem, discharge was averaged over the 5 s period immediately following the injection and expressed as a percentage of the pre-injection or control discharge averaged over 15–30 s. The maximum effect (—100%) was obtained when a drug caused a total abolition of discharge during the 5 s period, and the dose causing a 50% reduction (ID_{50}) was calculated from dose–response lines. The mean (± S.E.M.) ID_{50} values obtained were 4.2 ± 1.8 nmol for dopamine (n = 6 experiments), 3.3 ± 1.3 nmol for LY 141865 (n = 3) and 150 nmol for SK & F 38393 from the two experiments in which values could be obtained — in one experiment an ID_{50} could not be determined because even very high doses of SK & F 38393 caused only slight decreases in chemoreceptor discharge.

Dopamine receptor antagonists

The effects of domperidone on chemoreceptor activity were investigated in 5 experiments; (—)-sulpiride was studied in a single experiment. A dose of 10 $\mu g \cdot kg^{-1}$ i.c. was used because it exceeded the threshold dose of domperidone (0.1–1 $\mu g \cdot kg^{-1}$) needed for antagonism of dopamine-induced chemodepression, but was not high enough to block completely the responses to all doses studied, as occurred after 200 $\mu g \cdot kg^{-1}$. Domperidone greatly reduced the chemo-inhibitory effect of dopamine (Fig. 6) and also antagonized the effects of LY 141865 and SK & F 38393. The shift to the right in the dose–response lines can be clearly seen in Fig. 7. No consistent increases in chemosensory activity occurred in response to injections of dopamine or LY 141865 fol-

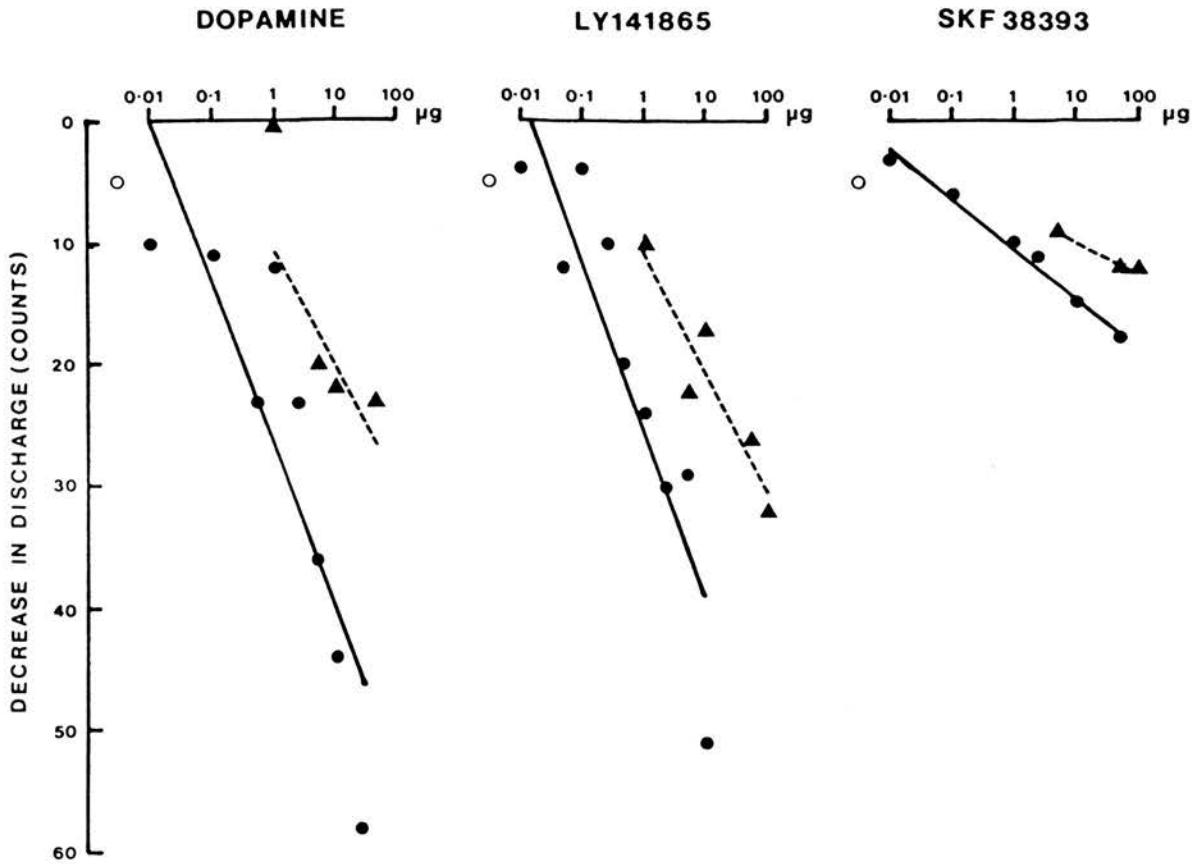

Fig. 7. Data obtained from a recording of chemoreceptor activity in one rabbit in which the depression of discharge observed following intra-carotid injection of dopamine, LY 141865 and SK & F 38393 has been plotted against the dose injected ($\log_{10}$ scale), and lines fitted to the data by the method of least-squares. Filled circles and solid lines are responses obtained pre-domperidone, filled triangles and dotted lines those after domperidone, 10 $\mu g \cdot kg^{-1}$ i.c. The decrease in discharge ($-\Delta\Sigma\chi$) is the difference between the number of action potentials counted during the period when discharge was depressed, and the number anticipated in that period had discharge continued at the pre-injection frequency (see Docherty and McQueen[8]). The open circles show the average effect of injecting the drug vehicle and wash solution (respectively 0.1 ml unbubbled Locke solution + 0.2 ml Locke solution which had been bubbled with 5% CO_2–95% air in a water bath at 37 °C). The rabbit was artificially ventilated with air and analysis of arterial blood samples showed there was no significant change in blood gas tensions or pH after domperidone. Background chemoreceptor discharge (average of all he pre-injection control values ± S.E.M.) was 4.9 ± 0.4 cps before and 8.8 ± 0.5 cps after domperidone ($P < 0.01$).

lowing treatment with domperidone.

The antagonism of dopamine-induced chemodepression lasted for at least 3 h following a single dose of domperidone and this meant that studies, in addition to those already described, could be undertaken in most of the experiments. Responses to suprathreshold submaximal doses of sodium cyanide, Locke solution equilibrated with CO_2, were obtained and expressed as the percentage change from control responses obtained predomperidone. Similarly, the effects of domperidone on background (spontaneous) activity and on the discharge recorded at the peak of the response to breathing 10% O_2–90% N_2 were determined. The results, summarized in Fig. 8, showed that background activity was variably affected by domperidone, there being an increase in discharge on average, whereas responses to hypoxia, cyanide and CO_2 were reduced. (—)-Sulpiride (10 $\mu g \cdot kg^{-1}$ i.c.) had effects on responses to dopamine, LY 141865, SK & F 38393, hypoxia and cyanide which were very similar to those of domperidone.

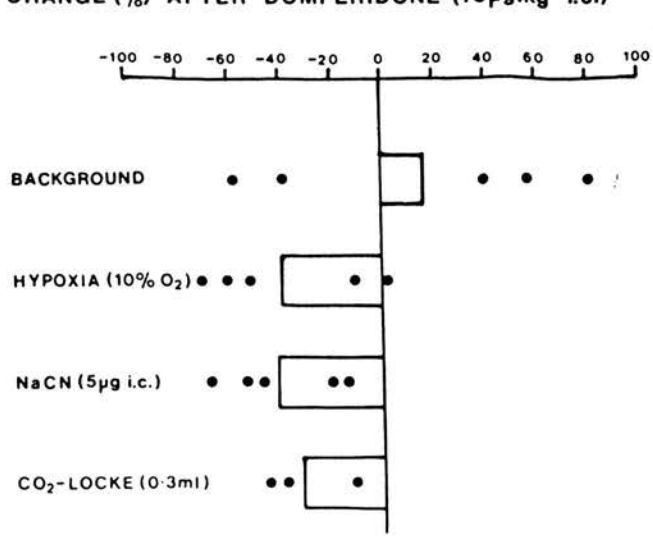

Fig. 8. The percentage changes in background discharge and in the responses of carotid chemoreceptors to hypoxia, sodium cyanide, and CO_2-equilibrated Locke solution after domperidone (10 $\mu g \cdot kg^{-1}$ i.c.) are shown. Each point gives the data obtained from an individual experiment, and the open bars represent averaged changes from the pre-domperidone values.

DISCUSSION

A widely accepted classification of dopamine receptors, first proposed by Kebabian and Calne[18], subdivides receptors into those that are positively linked to adenylate cyclase (D_1) and those that are not associated with this enzyme (D_2). More recently, there is growing evidence that at least in some tissues D_2 receptors may suppress cyclic AMP formation[6]. It is from this framework that we have attempted to classify dopamine receptors in the rabbit carotid body, since not only does there appear to be a different biochemical effector response associated with each subtype, but there are a growing list of relatively selective agonists and antagonists to use in functional studies. It was therefore gratifying that the present results from biochemical and neuropharmacological studies, provide strong evidence for the presence of functional dopamine D_2 receptors in the rabbit carotid body.

Biochemical studies

In our initial attempts to label dopamine receptors in carotid body homogenates, we used the high affinity ligand, [^{3}H]spiperone. However, in view of evidence that this neuroleptic can label $5HT_2$ receptors in the central nervous system[16,33] and the indication that the binding displaced in the carotid body by the specific D_2-antagonist, sulpiride, was less than that displaced by butaclamol (which has high affinity for both dopamine D_2 and $5HT_2$ receptors), persuaded us to perform all further studies with the selective D_2 ligand, [^{3}H]domperidone[20]. It should be noted that Dinger et al.[7] first reported specific binding of [^{3}H]spiperone to intact rabbit carotid bodies, though it is not know what proportion of dopamine D_2 and 5-HT_2 receptors were labelled in that study.

Although the lack of sufficient tissue precluded a detailed pharmacological analysis of the [^{3}H]domperidone sites in the carotid body, saturation analysis clearly demonstrated a homogeneous population of sites that possessed an affinity that was very similar to that found in the rat striatum where a very detailed pharmacological study has been completed[20]. Domperidone has high specificity for D_2 receptors and its high affinity for carotid body sites precludes association with other receptors[20].

The absence of functional dopamine D_1 receptors is strongly indicated by the inability of dopamine to stimulate adenylate cyclase in homogenates or cyclic AMP accumulation in intact carotid bodies. This data confirms earlier studies in the cat by Fitzgerald et al.[12] and, in view of the effectiveness of fluoride in broken cells and isoprenaline in the intact preparation, also suggests that this negative finding does not relate to an uncoupling of D_1 sites during preparation of carotid body homogenates or the inability of the preparation to respond to agonist stimulation. It should also be noted that dopamine did not produce any significant inhibition of control cyclic AMP formation in the homogenates or cyclic AMP content in intact tissue.

In an attempt to localize dopamine receptors in the carotid body, Dinger et al.[7] reported that chronic section of the carotid sinus nerve reduced specific [^{3}H]spiperone binding by 64%. It should be emphasized, however, that under the conditions of their assay, it is not clear what proportion of dopamine D_2 or $5HT_2$ receptors are being labelled, and it is significant that in the present study an identical denervation procedure reduced the binding of the specific D_2 ligand, [^{3}H]domperidone by only 32%. This may suggest that there are both dopamine D_2 and possibly

$5HT_2$ receptors on carotid sinus nerve terminals. In the present context, it is also important to note that the majority, at least, of D_2 sites may be present on cells (Type I and II), and it will be important to delineate their function (autoreceptors regulating transmitter synthesis and release?).

Neuropharmacological studies

Depression of chemosensory discharge was caused by dopamine and by the selective D_2-receptor agonist, LY 141865. The drugs were approximately equipotent, whether expressed in terms of the overall reduction in discharge ($-\Delta\Sigma\chi$) or as the ID_{50}. The latter index is based on the response in the 5 s post-injection period and has the advantage of excluding drug-induced effects that might indirectly influence chemoreceptor activity. In contrast, the D_1-receptor agonist, SK & F 38393, was very much less effective in depressing discharge. The effects of dopamine and LY 141865 were antagonized by the D_2-receptor antagonists, domperidone and (—)-sulpiride, and the fact that domperidone also antagonized the effect of SK & F 38393 suggests that this agonist at high concentration can act on dopamine D_2-receptors[29], but not very effectively. Thus, the evidence obtained using the selective dopamine D_2-receptor agonist and antagonists, coupled with the lack of effect of the D_1-agonist, even after antagonism of the D_2-receptors, suggests that the receptor mediating dopamine-induced chemodepression can be classified as being a D_2 type, and that dopamine D_1-receptors are either not present in the carotid body (see above) or do not influence chemoreceptor discharge significantly under the conditions of our experiments. Zapata et al.[39] have also concluded that chemo-inhibition is mediated by a dopamine D_2-receptor in the cat carotid body.

Previous studies in rabbits in vivo have shown that dopamine-induced chemodepression is antagonized by α-flupenthixol[9] or haloperidol[13], drugs which are D_2-receptor antagonists. However, although these results can be interpreted as supporting our present conclusions regarding the presence of D_2-receptors in the carotid body, caution is needed because these antagonists also have effects on other pharmacological receptors[32].

Studies on the in vitro preparation of the rabbit carotid body[28] have shown a chemo-excitatory action of dopamine, and Folgering et al.[13] also found such an effect in vivo following administration of haloperidol. We failed to find evidence for any consistent chemo-excitation in response to injections of any of the dopamine receptor agonists studied either before or after domperidone. The differences between results obtained from in vivo and in vitro carotid bodies may be attributed to fundamental dissimilarities in the experimental preparations being studied. The delayed increase in chemosensory discharge observed by Folgering et al.[13] could be blocked by the β-antagonists, propranolol and metoprolol, thereby implicating a β-adrenoceptor. However, the increase in chemoreceptor discharge observed in dogs[3,4] was antagonized by D-tubocurarine (not acting as a nicotine antagonist) and not by propranolol. It is difficult to know how to interpret the evidence concerning the excitatory actions of dopamine, but the fact that we failed to detect such an effect under our experimental conditions, in which the ganglioglomerular nerves were cut and the animals were normoxic and normocapnic, may mean that much depends on the experimental conditions and techniques used by different workers.

Chemoreceptor responses to hypoxia (steady-state discharge), sodium cyanide and CO_2 were generally reduced after a dose of domperidone which significantly attenuated the chemo-inhibition evoked by exogeneous dopamine. This could be taken as evidence for endogeneous dopamine, acting via dopamine D_2-receptors, being involved in chemo-excitation, but more detailed studies using a wider dose-range for the antagonist(s) are needed to investigate this possibility, particularly since the response to cyanide is potentiated after α-flupenthixol[9], as is the effect of hypoxia after haloperidol[13] in rabbits. It should also be emphasized that there is no reason to accept that exogeneous dopamine agonists and antagonists delivered via the carotid artery will interact with the same receptors as endogeneously released dopamine which may be excitatory[11]. The important point to be made is that the functional activity of rabbit carotid body chemoreceptors is not grossly impaired by D_2-receptor antagonists under the present experimental conditions.

The present work does not establish where in the receptor complex the dopamine D_2-receptors are located. The ligand binding results suggest some are associated with carotid sinus nerve endings, and Okaji-

ma and Nishi[30] have presented evidence from experiments in cats, in which the carotid body is made ischaemic for 1 h, showing that chemo-inhibition in response to dopamine is still obtained when blood flow through the organ is re-established, but excitation no longer occurs. They interpret the results as meaning that chemo-inhibition results from actions on nerve endings, which, it is claimed, are relatively unaffected by ischaemia, and that chemo-excitation results from actions on glomus (Type I) cells which, it is presumed, undergo degeneration during ischaemia. However, whether ischaemia of the carotid body really produces such selective effects on the Type I cells seems a moot point. As far as actions on the cells are concerned, Matsumoto et al.[21] recorded intracellular potentials from rabbit carotid body Type I cells in vitro and found that dopamine (30–100 μg) depolarized some cells, but caused hyperpolarization followed by depolarization in others, and some cells showed no change at all in resting membrane potential in response to dopamine.

So, the questions of what the physiological role of dopamine is, the precise role and function of dopamine D_2-receptors, and the relationship between dopamine and other putative transmitters present in the carotid body (e.g. noradrenaline, enkephalins, substance P) remain to be established.

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INCREASED SENSITIVITY OF RABBIT CAROTID BODY CHEMORECEPTORS TO DOPAMINE AFTER CHRONIC TREATMENT WITH DOMPERIDONE

DANIEL S. MCQUEEN *,‡, ANIS K. MIR ***,†, HARRY M. BRASH ** and STEFAN R. NAHORSKI ***

*Departments of * Pharmacology and ** Medicine, University of Edinburgh Medical School, Edinburgh EH8 9JZ, and *** Department of Pharmacology and Therapeutics, University of Leicester Medical School, Leicester LE1 7RH, U.K.*

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An increase in specific dopamine D_2 receptor binding sites was observed in membranes prepared from the carotid bodies of rabbits treated for 8 weeks and then withdrawn for 4-9 days from the D_2 antagonist domperidone (2-5 mg/kg per day). Recordings of chemoreceptor afferent discharge from the carotid body also revealed that this change in receptor density was accompanied by an increased sensitivity to the chemodepressant effects of exogenous dopamine. The chemoreceptor responsiveness of the carotid body to hypoxia is blunted in rabbits treated chronically with domperidone, but this can be restored to normal by an acute dose of the D_2 antagonist. These experiments provide evidence that is compatible with a chemo-inhibitory role for endogenous dopamine in the rabbit's carotid body. Furthermore, these results suggest that the carotid body provides a useful model for the functional studies of dopamine D_2 receptors.

Dopamine D_2 receptors Carotid body Chronic domperidone treatment Hypoxia

1. Introduction

Dopamine is known to be present in the carotid body of various species, including the rabbit (Chiocchio et al., 1966; Dearnaley et al., 1968; Mir et al., 1982). The findings that exogenous dopamine can modify chemosensory discharge (Black et al., 1972; Sampson, 1972; Zapata, 1975; Docherty and McQueen, 1979) and that dopamine is released from the carotid body during exposure to hypoxia in vitro (Fidone et al., 1982) have led to suggestions that this catecholamine may be a modulator of chemosensory discharge (see Eyzaguirre and Fidone, 1980).

The predominant effect of dopamine when injected close-arterial to the rabbit carotid body in situ is a depression of 'spontaneous' or background chemosensory discharge (Docherty and McQueen, 1979) and evidence has been obtained (Mir et al., 1983) which suggests that this is mediated via a dopamine D_2 receptor, according to the classification proposed by Kebabian and Calne (1979).

The present study was undertaken in order to provide further information on the possible role of dopamine in the carotid body and, in particular, to investigate whether 'supersensitivity' of the dopamine receptor mediating chemodepression occurs following chronic treatment with a dopamine receptor antagonist, such as occurs at certain sites in the central nervous system after chronic administration of neuroleptics (e.g. see Rupniak et al., 1983).

Domperidone, a selective dopamine D_2 receptor antagonist, was used since it is effective in antagonising the chemodepressant effect of dopamine in the rabbit (Mir et al., 1983) and does not readily cross the blood-brain barrier (Laduron and Leysen, 1979). Hopefully, this property should

† Present address: Merrell International, Strasbourg, France.
‡ To whom all correspondence should be addressed.

preclude complications arising from indirect effects on the carotid body resulting from central dopamine receptor blockade. In the present study, a combination of biochemical, neuropharmacological and neurophysiological techniques was used to study carotid body dopamine receptors in the rabbit after a period of chronic domperidone treatment. Preliminary extracts of some of these results have been published (McQueen et al., 1983; McQueen, 1984).

2. Materials and methods

2.1. Animals

Experiments were performed on male New Zealand white rabbits weighing 2.5-4.4 kg, median weight 3.3 kg, given food and water ad libitum and kept under circadian illumination.

2.2. Drug dosage

Domperidone was administered for 8 weeks to one group of rabbits by adding the drug to drinking water so that the daily dose averaged 1-5 mg/kg. Domperidone was dissolved in acid and diluted with tap water to stock solution containing 5 mg/100 ml. A second group of rabbits which had the drug vehicle added to their drinking water served as controls. Average intake of rabbits was 300 ml/day. Animals were used for experiments 4-9 days after cessation of treatment.

2.3. Biochemical assays

The surgical procedures for the dissection of the carotid body were as previously described (Mir et al., 1982). Preparation of washed carotid body membranes free of contaminating dopamine and assay of specific [^{3}H]domperidone binding has been described in detail (Mir et al., 1983). In brief, washed membranes from two carotid bodies were incubated with [^{3}H]domperidone (0.4 nM) in 2 ml 50 mM Tris-HCl buffer, pH 7.6, for 30 min at 22°C. Membranes were then harvested on glass fibre (Whatman GF/C) filters, washed rapidly and counted for radioactivity. Specific binding was defined as binding displaced by 5 μM d-butaclamol. An analysis of plasma levels of domperidone was performed using a radioreceptor assay, slightly modified from that described by Creese and Snyder (1977). The content of noradrenaline, dopamine and 5-hydroxytryptamine was assayed using high performance liquid chromatography coupled to electrochemical detection, as previously described (Mir et al., 1982) except that a reverse phase/ion pair 5 μ Ultrasphere column was used for amine separation.

2.4. Recording of chemosensory discharge

Rabbits were anaesthetised with pentobarbitone sodium (35 mg/kg i.v. supplemented by 10% of the initial dose every 1-2 h). The trachea was cannulated and the animal artificially ventilated with air and paralysed by gallamine triethiodide (3 mg/kg i.v.). Arterial blood pressure was recorded from one femoral artery and the other artery was cannulated and used for withdrawing samples of arterial blood for analysis. A femoral vein was also cannulated for intravenous administration of drugs.

Electrical activity from filaments dissected from the peripheral end of the centrally cut sinus nerve was recorded using bipolar platinum/iridium electrodes, amplifiers, oscilloscope and FM tape-recorder, as previously described (McQueen, 1977). The ipsilateral ganglioglomerular (sympathetic) nerves were cut, and a fine catheter inserted into the common carotid artery until its tip was positioned about 1 cm caudal to the carotid bifurcation. Drugs were injected close-arterial (i.c.) to the carotid body from which activity was being recorded by injecting into the lingual catheter (over a 1-2 s period) 0.1 ml of drug solution together with a wash consisting of 0.2 ml Locke solution which had been equilibrated with 5% CO_2 : 95% air at 37°C. There was an interval of 5 min between successive injections.

Changes in PaO_2 were achieved by altering the inspired gas from air to either (a) 10% O_2 : 90% N_2, (b) 100% N_2, or (c) 100% O_2. Arterial blood gas tensions and pH were measured at equilibrium in samples of femoral arterial blood using a Radiometer BMS 3 analyser.

2.5. Analysis of neural activity

The taped signal was replayed through an oscilloscope linked to a pulse-height (window) discriminator (WPI 120) which was used to select chemoreceptor action potentials for numerical analysis. The output pulses from the discriminator represented the summed activity of all the action potentials discriminated (generally from 1-4 different chemoreceptor units) and these were counted at 0.1 s intervals over periods ranging from 60 to 480 s by a custom-built counter/timer linked to a Commodore 3032 microcomputer. The accumulated counts for each interval were read directly through the digital 'User Port' interface of the computer, displayed on the screen, and subsequently recorded on disk (Commodore 3040 disk drive). The computer was also used to calculate, from the data stored on disk, the response of the chemoreceptor unit(s) to a drug or procedure in terms of, for example, mean discharge frequency ($\bar{x}$ ct/s) or the integrated discharge (Σx counts) and a printout of such variables obtained (Commodore printer 4022).

2.6. Statistical tests

Statistical comparison of data was made using either Student's t-test or, where sample sizes permitted, the Wilcoxon two-sample test. Mean values are shown $\pm$ S.E.M., and differences between means were regarded as statistically significant when $P \leqq 0.05$.

2.7. Drugs

Dopamine hydrochloride (mol. mass 190, Sigma); [^{3}H]domperidone (59.9 Ci/mmol, New England Nuclear); domperidone and ketanserin were kindly donated by Janssen Pharmaceuticals; LY-141865 (mol. mass 292, kindly donted by Eli Lilly & Co. Ltd.); d-butaclamol (Ayerst).

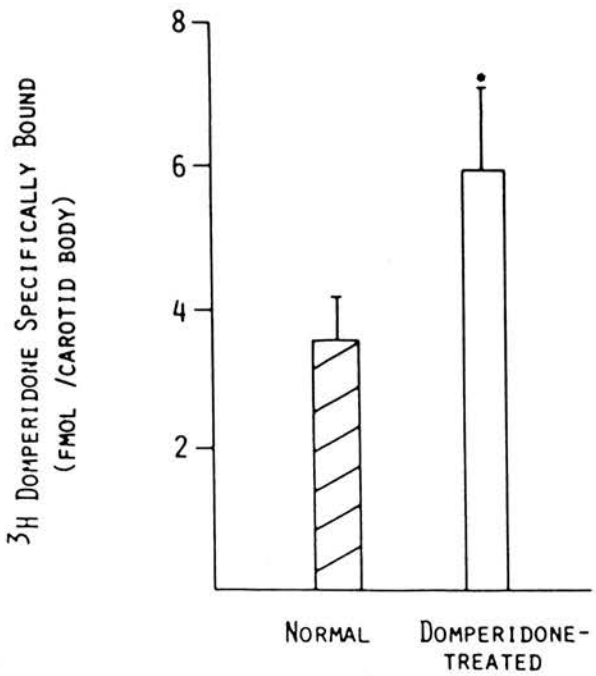

Fig. 1. Comparison of mean specific [^{3}H]domperidone binding (fmol bound/carotid body $\pm$ S.E.M.) to rabbit carotid body membranes in vehicle-treated rabbits and those which had been treated chronically with domperidone. * Statistically significant ($P < 0.05$, $n = 4$).

3. Results

3.1. Effects of chronic treatment with domperidone on binding of [^{3}H]domperidone to carotid body membranes

The chronic administration of domperidone to the rabbits was achieved by providing the drug in the drinking water. The intake of drinking water was monitored daily (average about 300 ml/day) and this provided a daily dose of the D_2 antagonist of 2-5 mg/kg per day. Radioreceptor assay of plasma domperidone in 3 rabbits at 6 weeks after commencement of dosing gave a range 38-83 ng/ml plasma of domperidone equivalents (see Creese and Snyder, 1977). Although we do not know of comparable studies performed with this drug in rabbits, the plasma levels are similar to those reported 4 h after oral dosing of 40 mg domperidone to man (Heykants et al., 1981). This dosing schedule did not overtly alter the spontaneous behaviour of the animals.

The specific binding of [^{3}H]domperidone to washed membranes of carotid bodies was significantly greater in rabbits that had been treated

chronically with the D_2 antagonist than in vehicle-treated rabbits (fig. 1). In these experiments, because of the extreme paucity of membranes available, binding of the ligand was performed only at a single, though saturating (0.4 nM) concentration of [^{3}H]domperidone (see Mir et al., 1983). We would argue, however, that our data closely reflects the maximal number of binding sites and if any residual domperidone was still present in the membranes, this would result in a decrease rather than the observed increase in specific binding.

In a few experiments, striatal membranes were also examined from both domperidone and vehicle-treated rabbits. The specific binding of [^{3}H]domperidone was unaltered by chronic treatment (data not shown) emphasising the inability of this D_2 antagonist to cross the blood-brain barrier.

Analysis of noradrenaline, dopamine and 5-hydroxytryptamine in the carotid bodies of 4 control and 5 domperidone-treated animals revealed no significant difference between the groups in the levels of individual amines.

3.2. Effects of chronic treatment with domperidone on responses of chemoreceptors to dopamine and LY-141865

Dopamine caused chemodepression when injected close-arterial to the rabbit carotid body in doses of 0.01-50 μg i.c., and an index of chemoreceptor sensitivity to dopamine was derived by calculating the ID_{50}, i.e. the dose of dopamine causing a 50% reduction from mean 'spontaneous' or pre-injection chemosensory discharge level during the 5 s period immediately following the injection, as illustrated in fig. 2. Data obtained from different experiments were pooled and it was found that the average ID_{50} for dopamine was significantly lower in rabbits that had been treated chronically with domperidone (0.81 ± 0.23 nmol, $n = 6$), in comparison with vehicle-treated controls (3.8 ± 0.9 nmol, $n = 13$; $P < 0.01$). Background or pre-injection chemosensory discharge averaged 11.1 ± 2.5 ct/s in controls and 12.5 ± 4.9 ct/s in chronically treated rabbits, values that are not significantly different ($P > 0.05$). There was no

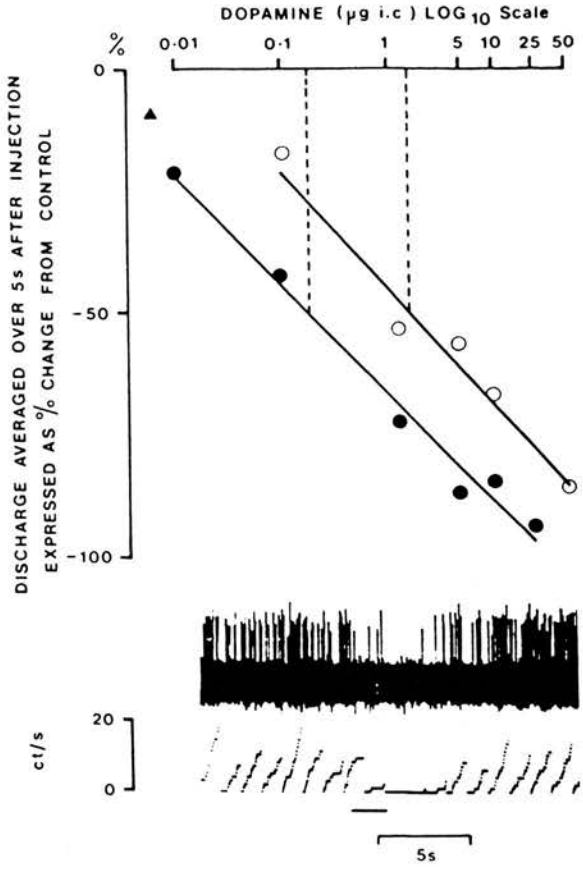

Fig. 2. Semi-log plot of dopamine injected i.c. against the depression of chemosensory discharge observed in the 5 s period immediately following the injection in a rabbit that had been treated chronically with domperidone (●———●). A straight line was fitted to the data by the method of least squares and the ID_{50} calculated (0.18 μg–0.95 nmol, dotted line). In contrast (○———○), an ID_{50} of value of 1.78 μg (9.4 nmol) was obtained from a rabbit that had been given only a single dose of domperidone (5 mg kg^{-1} s.c.) 8 days before the experiment. The effect of injecting the drug vehicle, Locke solution, is also shown (▲). The neurogram in the lower part of the figure illustrates the effect of dopamine (1.25 μg; 6.6 nmol i.c. at bar) in the chronically treated animal. The ramped trace is the cumulative output of the window discriminator, reset at 1 s intervals, and shows the response to dopamine of the 3 larger chemoreceptor units that were counted.

significant difference between ID_{50} values obtained from untreated (values obtained from re-analysis of previous experiments by Docherty and McQueen, 1979) and vehicle-treated rabbits. The dopamine D_2 receptor agonist LY-141865

(Tsuruta et al., 1981) gave ID_{50} values of 7.3 ± 1.9 (mean ±S.E.M.) nmol from experiments in 4 vehicle-treated rabbits, and 2.8 ± 2.1 nmol (n = 3) in animals that had undergone chronic domperidone treatment.

Administration of a single dose of domperidone (10 μg kg^{-1} i.c.) greatly reduced the chemodepressant effects of exogenous dopamine and LY-141865. After domperidone, the ID_{50} for dopamine increased by an averaged factor of 83 (n = 4) in vehicle-treated rabbits, and by 2900 (n = 4) in the case of animals which had been treated chronically with domperidone. Corresponding values for LY-141865 were 173 (n = 2) and 803 (n = 4). Overall background discharge increased from 8.2 ± 3.4 cps before the acute dose of domperidone to 12.3 ± 5.4 (n = 5) afterwards in vehicle-treated rabbits, and from 13.7 to 7.2 to 20.6 ± 9.3 (n = 4) in chronically treated rabbits. Background discharge increased by 44 ± 11.5% on average after the single dose of domperidone and the increase was statistically significant in 3 of the 5 vehicle-treated rabbits. The increase averaged 66 ± 25% in the 4 ex-chronic domperidone animals, and the rise was statistically significant in all 4 experiments. Mean background activity was not significantly different between the 2 groups, either before or after the acute dose of domperidone ($P > 0.05$).

3.3. Effects of chronic treatment with domperidone on responses of chemoreceptors to hypoxia

The responsiveness of the carotid body chemoreceptors to hypoxia was assessed by switching the inspired gas from room air to a hypoxic gas mixture (10% O_2 : 90% N_2) for 4 min and plotting chemosensory discharge against time. An equilibrium was reached after about 2 min when discharge plateaued (see fig. 3) and it was possible to fit a straight line to the rising part of the curve. The averaged plateau value was expressed as 100%, to normalise the data, and the slope of the line calculated. Discharge was submaximal on 10% O_2, and could be further increased by ventilating for 60 s with 100% N_2.

Values obtained for the slopes of the 'hypoxia lines' were pooled and are shown in fig. 4 where it

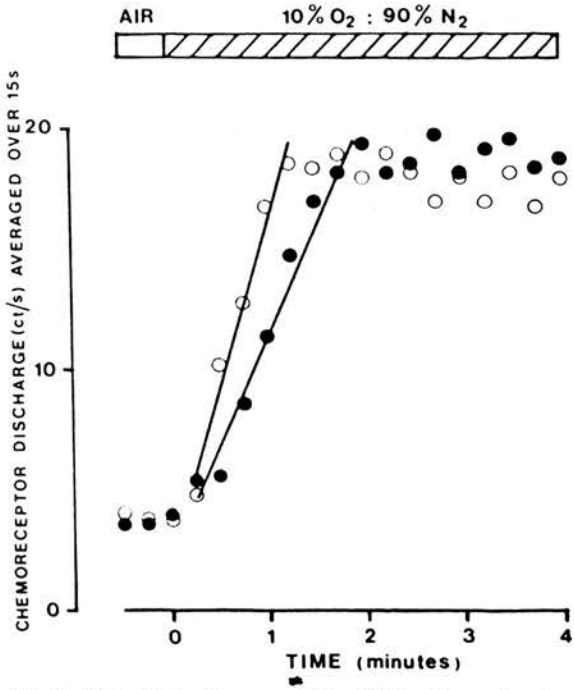

Fig. 3. Data obtained from a rabbit which had been chronically treated with domperidone (●———●) compared with that from a vehicle-treated control (○———○). Chemoreceptor discharge was averaged over 15 s intervals and plotted against time. Switching to ventilating with hypoxic gas (10% O_2) during the period shown caused an increase in frequency which plateaued after about 1-2 min and then remained steady about a mean level which was taken as maximum, or 100%. Straight lines were fitted by the least squares method to the values obtained during the period when discharge was increasing after switching to 10% O_2, and the slope of the lines expressed in terms of % maximum·s^{-1}. The slope was 0.8% max·s^{-1} ($r = 0.99$) and average steady state discharge (maximum) 19.0 ct/s in the chronically treated rabbit, whereas the slope was steeper (1.2% max·s^{-1}, $r = 0.99$; maximum = 18.2 ct/s) in the vehicle-treated control.

can be seen that the slope was significantly reduced in animals that had been treated chronically with domperidone, as compared with the vehicle-treated controls. Background and 'plateau' (i.e. on 10% O_2) discharge values were not significantly different between the groups. Administration of a single dose of domperidone (10 μg kg^{-1} i.c.) returned the slope towards control values (fig. 4).

Arterial blood samples were taken 3.5 min after changing from air to the hypoxic mixture in several experiments, and values obtained are shown in

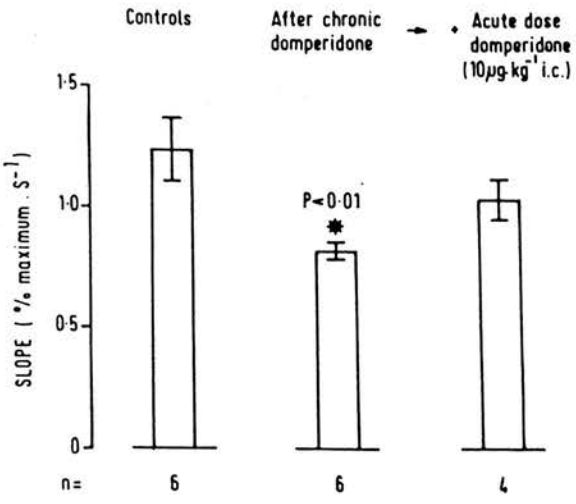

Fig. 4. Values for the slope of the line relating chemosensory discharge to time after switching to ventilating rabbits with the hypoxic gas mixture (10% O_2) are shown. In 4 of the rabbits which had been treated chronically with domperidone, after obtaining the response to hypoxia a single dose of domperidone was injected and the hypoxia test repeated. Background or 'spontaneous' discharge during ventilation with air was 4.4 ± 2.0 cps (mean $\pm$S.E.M.) for controls, 7.4 ± 2.1 cps for chronically treated rabbits, and 13.8 ± 3.1 cps in chronically treated rabbits after the single acute dose of domperidone. The 'plateau' or steady state discharge during ventilation with 10% O_2 averaged 25.2 ± 8.2 cps in controls, 31.6 ± 6.5 cps in chronically treated rabbits and 30.0 ± 9.7 cps in chronically treated animals after an acute dose of domperidone.

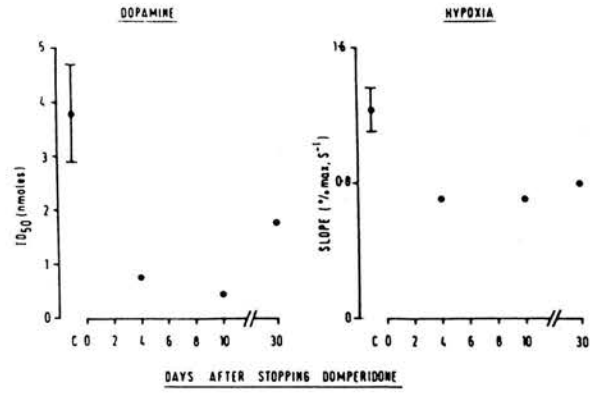

Fig. 5. Responses of chemoreceptors to dopamine (ID_{50} values) and hypoxia (slope of hypoxic response line) obtained in individual rabbits 4, 10, and 30 days after stopping the 8 week period of chronic treatment with domperidone (5 mg kg^{-1} daily). C shows the mean $\pm$S.E.M. control values from vehicle-treated rabbits 4-9 days after stopping treatment.

table 1. There were no major differences between groups, apart from the pH being significantly higher ($P < 0.05$) during hypoxia in ex-chronic domperidone rabbits cf. vehicle-treated.

3.4. Other experiments

Measurement of dopamine ID_{50} values and slopes of chemoreceptor discharge lines during hypoxia showed that, following treatment with domperidone for 8 weeks, values were decreased to similar extents during the 4-10 day period after withdrawing the drug, and even one month after stopping domperidone they had not returned to control levels (fig. 5).

The possibility that the effects observed were not attributable to the effects of chronic dosage with domperidone, but merely due to some residual traces of the drug, was tested by injecting a single dose of domperidone (5 mg kg^{-1} s.c.) into a rabbit and then 8 days later examining the chemoreceptor responses to dopamine and hypoxia. The ID_{50} for dopamine was found to be 9.4 nmol (see fig. 2), in the upper range of control values, and the slope of the hypoxia line was 1.22% max · s^{-1}, which was close to the average for control animals.

TABLE 1

Mean arterial blood gas tensions and pH $\pm$S.E.M.

	Normoxia				Hypoxia (10% O_2)			
	pH	$PaCO_2$ (torr)	PaO_2 (torr)	n	pH	$PaCO_2$ (torr)	PaO_2 (torr)	n
Controls	7.40 ± 0.02	32 ± 0.94	82 ± 3.2	14	7.35 ± 0.03	32 ± 2.2	48 ± 2.0	4
Domperidone-treated	7.44 ± 0.03	33 ± 1.9	72 ± 2.7	7	7.48 ± 0.04	30 ± 1.2	41 ± 6.6	3

4. Discussion

The results obtained from the present experiments using a combination of biochemical and neuropharmacological techniques can be interpreted as providing evidence that an increased sensitivity of dopamine D_2 receptors occurs in the rabbit carotid body following a period of chronic treatment with domperidone. Furthermore, this altered responsiveness appears to accompany an increased number of specific D_2 binding sites in this tissue. The strength of this combined approach is highlighted in that the present data provides the first quantitative functional evidence that an elevation of dopamine D_2 receptors is probably associated with an increased sensitivity of cells to both exogenous and endogenous dopamine.

Although there has been a large number of studies in the central nervous system that demonstrate increased numbers of dopamine receptor binding sites following chronic neuroleptic administration, functional consequences of this phenomenon have been largely limited to indirect measures of altered behaviour (see Rupniak et al., 1983). However, Gallagher et al. (1978) have reported that low doses of dopamine agonists cause a greater depression of dopamine cell firing after chronic haloperidol, this presumably reflecting an increased responsiveness of somatodendritic dopamine autoreceptors. In the present study it is by no means certain at which sites dopamine receptors are elevated. Previous studies from this laboratory (Mir et al., 1983) have revealed that 60-70% of specific D_2 sites are on (glomus) cells since they survive chronic denervation of the carotid body. Clearly in future studies it will be important to examine the effects of neuroleptic treatment in animals with denervated carotid bodies.

Exogenous dopamine depressed chemosensory discharge, as previously described (Docherty and McQueen, 1979; Mir et al., 1983), and responsiveness of the chemoreceptors in the present study has been expressed in terms of the ID_{50} of the drug so that a comparison could be made between different animals with widely varying background chemosensory discharge. The fact that the ID_{50} for dopamine was significantly lower in animals which had been treated chronically with domperidone suggests that the dopamine receptors mediating chemodepression may have become supersensitive. The increase in sensitivity to the D_2 agonist LY-141865 was not so great. Whether this relates to the partial agonist activity of this agent or to other phenomena requires further investigation.

In order to examine the responsiveness of the carotid body chemoreceptors to a physiological stimulus, namely hypoxia, it was necessary to devise some way of comparing chronically treated rabbits with the vehicle-treated controls, given that the number of chemoreceptor units recorded and their absolute discharge frequency in response to a fixed stimulus varied from experiment to experiment. It was decided to use the rate at which discharge increased on changing from ventilating the animal with air to the hypoxic gas mixture (10% O_2), a reproducible stimulus, and to normalise the slope of the line fitted to data points by expressing chemosensory discharge as a percentage of the plateau or mean steady-state discharge obtained in response to the hypoxic stimulus. The finding that the sensitivity of carotid body chemoreceptors to hypoxia was significantly reduced in rabbits treated chronically with domperidone, but could be restored towards normal by a single low dose of domperidone, provides evidence that endogenous dopamine can function as a depressant of chemosensory discharge (see Introduction) under the conditions of our experiments in which the carotid body was denervated. However, it is important to stress that it was only the rate at which chemosensory discharge increased in response to the hypoxic stimulus, and not the absolute discharge frequency eventually attained, which was significantly affected by chronic treatment with domperidone.

If we assume that similar alterations in carotid body dopamine receptor sensitivity may occur in man following long term dopamine antagonist treatment, then the functional consequences should be considered. Since the carotid body has little influence on ventilation under normal conditions, one could argue that in normal individuals little effect would be apparent. However, in those individuals with chronic obstructive ling disease, the blunting of sensitivity to hypoxia could potentially

compound their respiratory problem.

In conclusion, the present experiments have provided evidence for functional altered responsiveness of chemoreceptor activity to dopamine and increased dopamine D_2 receptor sites in the carotid body following chronic but not acute domperidone treatment. The blunted response to hypoxia in treated animals also supports the concept of a chemo-inhibitory role for endogenous dopamine in the carotid body.

Acknowledgements

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Changes in carotid body amine levels and effects of dopamine on respiration in rats treated neonatally with capsaicin

D.S. McQueen & A.K. Mir[1]

Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh 8 and Department of Pharmacology and Therapeutics, University of Leicester, University Road, Leicester 1

1 Dopamine levels in rat carotid bodies and the effects of intravenous dopamine injections on respiration in adult rats anaesthetized with pentobarbitone have been studied in animals which were treated with capsaicin neonatally.

2 Levels of dopamine were five fold higher in the carotid bodies of capsaicin-treated rats as compared with vehicle-treated controls, but there was no significant difference between capasicin-treated and vehicle-treated rats in their ID_{50} values for dopamine-induced respiratory depression.

3 Domperidone, a dopamine D_2-receptor antagonist, substantially reduced the respiratory depression caused by dopamine, both in capsaicin-treated and in control animals, suggesting that a D_2-receptor was involved in the response. Cutting the carotid sinus nerves greatly reduced the ventilatory-depressant effect of dopamine, showing that sensory receptors, most probably arterial chemoreceptors, were responsible for most of the response.

4 Substantially less reflex hyperventilation was evoked in capsaicin-treated rats by the peripheral chemoreceptor stimulants hypoxia and sodium cyanide, in comparison with the controls, and domperidone did not increase the responsiveness. About 80% of the reflex ventilatory change originated from carotid body chemoreceptors.

5 The hypoventilation caused by breathing 100% O_2 was not significantly different in capsaicin-treated rats when compared with controls. Domperidone substantially reduced this response in capsaicin-treated rats, but not in vehicle-treated animals.

6 Dopamine-induced respiratory depression in capsaicin-treated rats was slightly enhanced, rather than reduced, by oxygen breathing; domperidone remained an effective antagonist of dopamine-induced ventilatory depression.

7 Most of the reduction in respiration caused by dopamine in rats anaesthetized with pentobarbitone can be attributed to actions on a dopamine D_2-receptor located in the carotid body. However, despite the increased levels of dopamine found in the carotid bodies, the reduced peripheral chemosensitivity observed in anaesthetized capsaicin-treated rats does not appear to result from a change in sensitivity to dopamine.

Introduction

Reflex increases in respiration evoked by stimuli that activate peripheral arterial chemoreceptors are significantly reduced in anaesthetized adult rats which have been treated neonatally with capsaicin (Bond *et al.*, 1982), but the cause of this change in sensitivity remains to be established.

Dopamine depresses respiration in anaesthetized rats apparently by inhibiting arterial chemoreceptors (Hasan & Horn, 1980; Horn, 1981; Cardenas & Zapata, 1981; Horn *et al.*, 1984; Zapata *et al.*, 1984) and dopamine is present in rat carotid bodies and can be released from them during stimulation (Hellstrom, 1977; Hanbauer & Hellstrom, 1978). It seemed possible, therefore, that excessive dopamine release, or increased sensitivity of chemoreceptors to dopamine, might explain the reduction in peripheral chemosensitivity caused by capsaicin treatment. In

[1] Present address: Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg, France.

order to test this possibility two separate series of experiments were performed. Firstly, biochemical techniques were used to measure amine levels in carotid bodies taken from capsaicin-treated and from control rats. Secondly, a series of experiments was performed which involved recording ventilation in anaesthetized rats. The respiratory responsiveness to dopamine and domperidone, a dopamine D_2-receptor (see Kebabian & Calne, 1979) antagonist that blocks dopamine-induced chemodepression in cats and rabbits (McQueen, 1984; Mir *et al.*, 1984), of capsaicin-treated animals was compared with that of vehicle-treated controls. The sensitivity of the peripheral chemoreceptors to physiological and pharmacological stimuli was also tested in both groups.

Methods

Experiments were performed on Sprague-Dawley rats. Animals were anaesthetized with halothane (1% in O_2) on day 2–4 after birth and injected with either capsaicin (50 mg kg^{-1} s.c.) or drug vehicle (10% ethanol, 10% Tween 80 in saline) as previously described (Jancso *et al.*, 1977; Bond *et al.*, 1982). Three to eight months later they were used for experiments.

Estimation of amine levels in rat carotid bodies and superior cervical ganglia

Rats were anaesthetized with pentobarbitone (42 mg kg^{-1} i.p.) and their carotid bodies and superior cervical ganglia excised surgically as previously described (Mir *et al.*, 1982). Carotid bodies and ganglia were homogenized in 300 μl and 1000 μl respectively of 0.1 M ice-cold perchloric acid. The content of dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) was assayed using high performance liquid chromatography (h.p.l.c.) coupled to electrochemical detection, as previously described (Mir *et al.*, 1982) except that a reverse phase/ion pair 5 μ ultrasphere column was used for amine separation.

Respiratory experiments

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p., supplemented as required during the later part of some experiments). The level of anaesthesia, assessed qualitatively, was similar in the two groups but capsaicin-treated rats sometimes required a brief period (5–20 min) of artificial ventilation to keep them alive during the 30–60 min after injecting pentobarbitone. The trachea was cannulated as were both femoral arteries (one for measuring BP, the other for taking 300 μl arterial blood samples) and a femoral vein (for drug administration). During some of the experiments the carotid sinus nerves were identified and cut at their junction with the glossopharyngeal nerves and both vago-sympathetic nerves were cut in the neck.

Ventilation was measured with an integrating pneumotachograph, as previously described (McQueen, 1973). The animals breathed either room air, 100% O_2, or a hypoxic gas mixture (10% O_2: 90% N_2). The respiratory response to a hypoxic stimulus was obtained by switching from air to 10% O_2 for 3 min and taking an arterial blood sample at 2.5 min. Ventilation was measured 1 and 3 min after changing the inspired gas, and the same protocol was used for hyperoxia (100% O_2 inspired).

Drug administration

Doses of dopamine or sodium cyanide were injected intravenously (0.1 ml drug solution washed in with 0.2 ml Locke solution over 2–3s) with at least 5 min between successive doses. The respiratory volume (R.M.V.) during the 20s period (2 × 10 s ramps) immediately following the injection was calculated and expressed as a percentage change from the pre-injection (control) value and $\log_{10}$ dose-response lines plotted. Mean arterial BP was measured before and during the response period.

Statistical analysis

Mean values are given ± s.e. mean. Differences between groups were compared by using either the Wilcoxon two-sample test, the Wilcoxon signed ranks test (for paired data) or Student's paired *t* test (when there was insufficient data for the non-parametric test) and the null hypothesis rejected at P (2 tailed) < 0.05.

Drugs

Capsaicin (Sigma) was prepared as described above. Other drugs were dissolved in modified Locke solution (Docherty & McQueen, 1978): dopamine HCl (Koch-Light); sodium cyanide (B.D.H.); domperidone (kindly donated by Janssen Pharmaceuticals, Belgium).

Results

Amine measurements

Carotid bodies were excised from five capsaicin-treated rats (1 male 450 g; 4 females 256 ± 9.6 g) and from vehicle-treated controls (1 male 422 g; 4 female 249 ± 9.0 g) when the animals were four months old.

The amine levels in their carotid bodies, as estimated by h.p.l.c., are shown in Figure 1 and it was found that dopamine, noradrenaline and 5-HT levels were all significantly higher in capsaicin-treated rats compared with the controls ($P<0.05$). There was no significant difference between amine content of left and right carotid bodies within either group.

Superior cervical ganglia (SCG) from capsaicin-treated rats also had significantly higher dopamine levels (215 ± 8.5 pmol per SCG, $n=9$, $P<0.05$) than the controls (60.3 ± 1.7 pmol per SCG, $n=7$), but noradrenaline levels in capsaicin-treated animals (142 ± 2.3 pmol per SCG, $n=9$) were not significantly different from controls (142 ± 12 pmol per SCG, $n=7$, $P>0.05$).

Respiratory responses to dopamine

Dopamine (5.3–105 nmol) caused a dose-dependent depression of ventilation (V) when injected i.v. in capsaicin-treated and in vehicle-treated rats (Figure 2). The maximum reduction that could be obtained during the 20 s post-injection period varied from animal to animal, but averaged $55 \pm 4\%$ in vehicle-treated rats and $63 \pm 4\%$ in the capsaicin-treated group ($P>0.05$). The dose causing half-maximal depression of ventilation (ID_{50}) was calculated for each experiment (e.g. Figure 3) and averaged

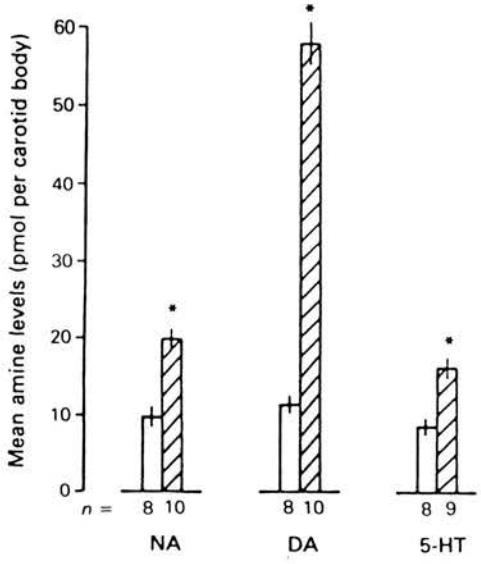

Figure 1 Amine levels, estimated by h.p.l.c., in carotid bodies of vehicle treated (open columns) and capsaicin-treated rats (hatched columns). Vertical lines show s.e.mean. n = number of carotid bodies. *$P<0.01$ (Wilcoxon two sample test). NA = noradrenaline, DA = dopamine; 5-HT = 5-hydroxtryptamine.

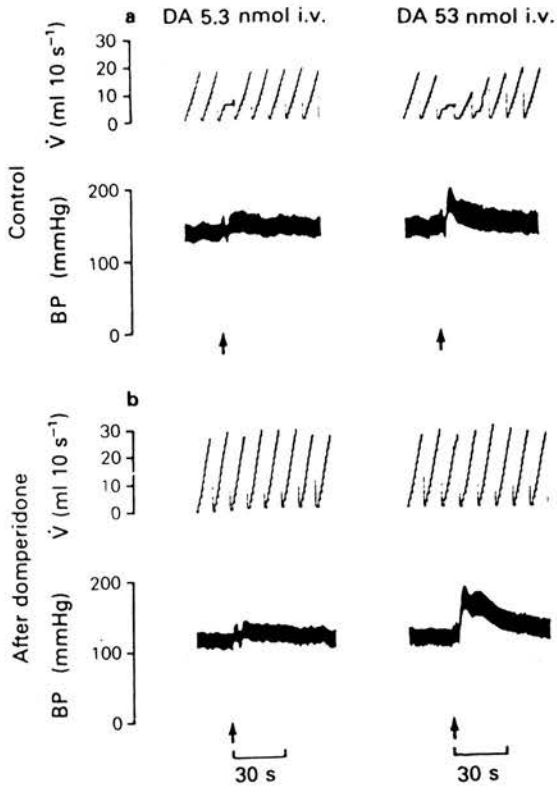

Figure 2 Respiratory (V) and blood pressure (BP) responses to two doses of dopamine (DA; 5.3 and 53 nmol i.v. at arrows) in an anaesthetized capsaicin-treated rat (a) before and (b) after administering the dopamine D_2- receptor antagonist domperidone (100 μg kg^{-1} i.v.). The respiratory trace reads from bottom to top with each step representing a single breath and the total ramp height showing the volume breathed in 10 s. Note that domperidone substantially reduced the hypoventilation caused by dopamine, but not the rise in blood pressure.

74 ± 14 nmol kg^{-1} (range 26–141, $n=8$) in controls and 108 ± 24 nmol kg^{-1} (range 28–287, $n=13$) in capsaicin-treated rats. Average ventilation pre-injection was 30.6 ± 2.4 ml 100 g^{-1} min^{-1} in the former and 32.0 ± 2.3 ml 100 g^{-1} min^{-1} in the latter group. None of these values was significantly different between groups. Blood pressure changes were minimal with lower doses of dopamine, but higher doses caused a dose-related hypentesion (e.g. Figure 2). The mean rise in BP associated with the 26 nmol dose of dopamine was 7 ± 5.5 mmHg in controls ($n=8$) and 12 ± 3.6 mmHg in capsaicin-treated rats ($n=13$; $P>0.05$).

The dopamine antagonist domperidone (100 μg kg^{-1} single dose) greatly reduced the respirat-

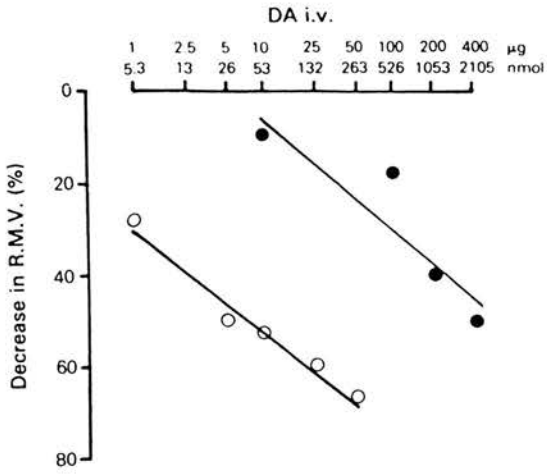

Figure 3 Dose-response plot of the respiratory responses to dopamine (DA) obtained during the experiment shown in Figure 2 before (○——○) and after (●——●) domperidone (100 μg kg^{-1} i.v.) in a 286 g capsaicin-treated female rat. Straight lines were fitted to the data by the least squares method and the dose required for half-maximal response (ID_{50}) calculated. The ID_{50} was 28 nmol kg^{-1} before domperidone, and 2611 nmol kg^{-1} for the same response after the antagonist. Mean ventilation (R.M.V.) averaged 39 ± 1.6 ml 100 g^{-1} min^{-1} pre-domperidone and 59.4 ± 1.7 ml 100 g^{-1} min^{-1} post-domperidone.

ory depressant effect of dopamine (see Figures 2 and 3) and the corresponding ID_{50} values obtained after the antagonist were 2300 ± 660 nmol kg^{-1} for controls ($n = 7$) and 3350 ± 990 nmol kg^{-1} for capsaicin-treated rats ($n = 11$). The ID_{50} values were significantly higher after domperidone but the difference between groups was not significant. Steady-state ventilation was 31.7 ± 2.5 ml 100 g^{-1} min^{-1} in control rats before domperidone and 37.8 ± 5.1 ml 100 g^{-1} min^{-1} after domperidone ($n = 7$; $P > 0.05$). capsaicin-treated rats ventilation increased significantly from 32.6 ± 2.7 ml 100 g^{-1} min^{-1} to 40.7 ± 3.0 ml 100 g^{-1} min^{-1} ($n = 11$, $P < 0.05$-Wilcoxon signed ranks test). Domperidone did not antagonize the pressor response to dopamine (e.g. see Figure 2).

Respiratory responses to hypoxia and hyperoxia

A reflex increase in ventilation occurred when rats breathed 10% O_2: 90% N_2 instead of air, and although the response was present in both groups, it was significantly greater in vehicle-treated rats (Figures 4 and 5). Measurement of the percentage increase in respiration 1 and 3 min after starting a 3 min period of hypoxia showed that hyperventilation was well-maintained in vehicle-treated rats, but not in the capsaicin-treated animals (Figure 5). Arterial blood gas tensions and pH values are shown in Table 1.

When 100% O_2 was breathed, respiration was reduced to about 40% of the value on air, and this decrease was not significantly different in the two groups.

Domperidone

Domperidone (100 μg kg^{-1} single dose i.v.) significantly reduced ($P < 0.05$; paired t test) the mean increase in ventilation evoked by 10% O_2 in controls (Figure 5) but although the response was also reduced in capsaicin-treated animals, with ventilation at 3 min now being depressed rather than increased, responses were variable and the differences from pre-domperidone mean values were not statistically significant ($P > 0.05$; paired t test).

In the presence of domperidone the reduction in respiration caused by breathing 100% O_2 was significantly attenuated in capsaicin-treated rats, but although not so well sustained in controls, the difference from pre-domperidone responses was not statistically significant.

Blood pressure values obtained before and during

Table 1 Mean arterial blood gas tensions and pH in rats breathing air, 10% O_2 or 100% O_2

		Vehicle-treated			*Capsaicin-treated*		
		Air	*10% O_2*	*100% O_2*	*Air*	*10% O_2*	*100% O_2*
Pa_{O_2} (KPa)	=	10.0 ± 0.35	5.87 ± 0.25	46.0 ± 3.2	9.70 ± 0.42	5.05 ± 0.35	28.1 ± 2.1*
Pa_{CO_2} (KPa)	=	4.67 ± 0.31	3.73 ± 0.47	6.80 ± 0.49	4.63 ± 0.17	3.47 ± 0.34	6.04 ± 0.55
pH	=	7.40 ± 0.02	7.47 ± 0.02	7.26 ± 0.04	7.39 ± 0.01	7.48 ± 0.03	7.28 ± 0.03
n	=	12	10	5	11	11	7

Samples were taken 1 min before and 2.5 min after switching from air to hypoxic or hyperoxic gas.
* $P < 0.05$ vs vehicle-treated. All other capsaicin-treated, not significantly different ($P > 0.05$) from corresponding vehicle-treated values.

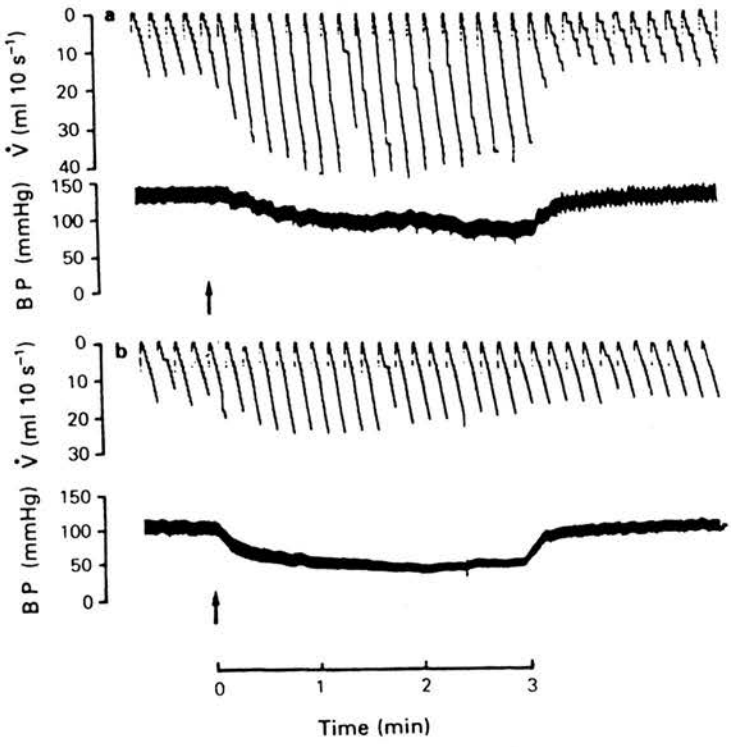

Figure 4 Respiratory and blood pressure responses to switching the inspired gas from room air to 10% O_2:90% N_2 for a 3 min period (commencing at arrow). (a) Vehicle-treated female rat (245 g). (b) Capsaicin-treated female rat (296 g) showing a much reduced and poorly sustained hyperventilation in response to the hypoxic stimulus as compared with the vehicle-treated control. The respiratory trace reads from top to bottom and interruptions at approximately 2 min intervals are caused by automatic amplifier resetting in the electrospirometer. Other details as in Figure 2.

hypoxia and hyperoxia, pre- and post-domperidone are shown in Figure 6. During 10% O_2 the mean BP in capsaicin-treated rats was significantly lower than in the vehicle- treated controls ($P<0.05$). After domperidone there was no statistically significant difference between groups. During oxygen breathing BP was significantly lower in capsaicin-treated rats compared with vehicle-treated controls ($P<0.05$). There was no significant difference in B.P. values pre- and post-domperidone measured during oxygen breathing in capsaicin-treated rats, but there was a small but significant difference ($P<0.05$, paired t test) between pre- and post-domperidone values from vehicle-treated animals.

Respiratory responses to sodium cyanide

NaCN was injected at two dose-levels, 1 and 4.1 μmol (50–200 μg) i.v. which produced responses that were suprathreshold but submaximal. Reflex hyperventilation was significantly greater in vehicle-treated controls than in capsaicin-treated rats (Figure 7). Domperidone reduced the ventilatory response to the high but not the low dose of cyanide in capsaicin-treated rats ($P<0.05$, paired t test), but in vehicle-treated rats the responses to either dose of cyanide before domperidone were not significantly different from the corresponding responses obtained after the antagonist.

Denervation experiments

In order to assess the relative contribution of different regions to the respiratory changes obtained, experiments were performed (mainly in vehicle-treated rats because capsaicin animals did not tolerate the procedure well) during which responses to dopamine, hypoxia, hyperoxia and cyanide were obtained before and after cutting the carotid sinus nerves. Subsequently, both vagosympathetic trunks were cut in the neck. In some experiments, the inverse sequence was followed, with the vagi being cut

first, and the carotid sinus nerves sectioned later in the experiment. Results obtained are summarized in Figure 8 which shows that cutting both sets of nerves totally abolished respiratory responses to hypoxia, cyanide and hyperoxia. Cutting the carotid sinus nerves, leaving the vagosympathetic trunk intact, reduced the ventilatory response to hypoxia by 83% (1st min), and the response was no longer well-sustained, ventilation being *depressed* by 20% at 3 min. Hyperoxia caused an increase in ventilation under these conditions, presumably reflecting removal of hypoxia-induced central respiratory depression, and the response to sodium cyanide was reduced by 80% compared with the intact preparation. Cutting the vagosympathetics abolished the residual responses to hypoxia and cyanide.

When the vagosympathetic trunks were cut first, leaving the carotid sinus nerve intact, the hyperventilation in response to hypoxia was only reduced by 16% and was well sustained. The response to hyperoxia was increased slightly and the response to cyanide reduced by 25% compared with the intact preparation. Cutting the carotid sinus nerves abolished the responses to hypoxia and sodium cyanide.

Cutting both sets of nerves substantially reduced the respiratory depressant action of dopamine obtained during air-breathing in vehicle-treated rats and most of the reduction could be attributed to the carotid sinus nerves since bilateral vagotomy alone did not greatly affect the response to dopamine. The effect of cutting both sets of nerves was thus rather similar to that obtained by administering domperidone.

Physiological denervation of arterial chemoreceptors was also attempted by ventilating the animal with 100% O_2 until a steady ventilatory state was reached. Responses to dopamine were studied during oxygen breathing and the respiratory changes measured. In vehicle-treated animals dopamine caused a depression of ventilation during oxygen breathing, but this was not a dose-related phenomenon. Domperidone reduced this response to dopamine, as it did during air breathing. In capsaicin-treated rats the dose-

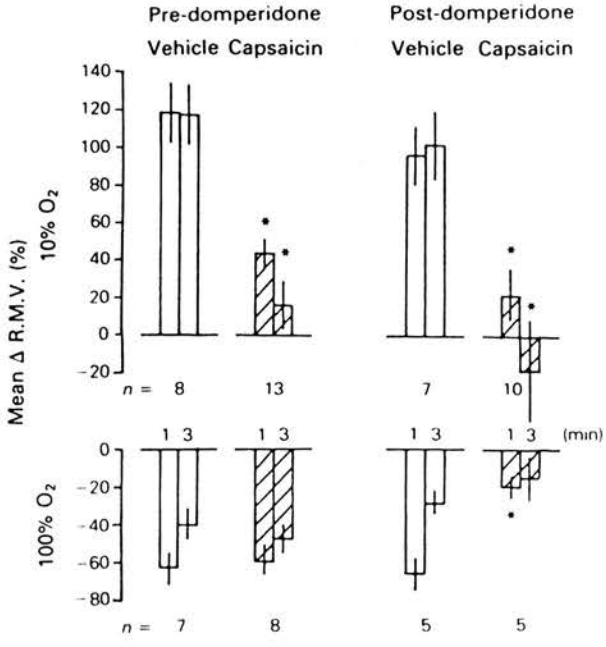

Figure 5 Mean changes in ventilation of vehicle-treated control (open columns) and capsaicin-treated (hatched columns) anaesthetized rats in response to switching from breathing room air to either 10% O_2 (upper panels) or 100% O_2 (lower panels) for 3 min Vertical lines show s.e. mean. Responses (1 and 3 min values shown for n animals) were obtained before and after injecting a single dose of domperidone (100 $\mu g\,kg^{-1}$ i.v.). $^*P<0.05$ in comparison with corresponding (i.e. pre- or post-domperidone) values from vehicle-treated rats using the Wilcoxon two sample test.

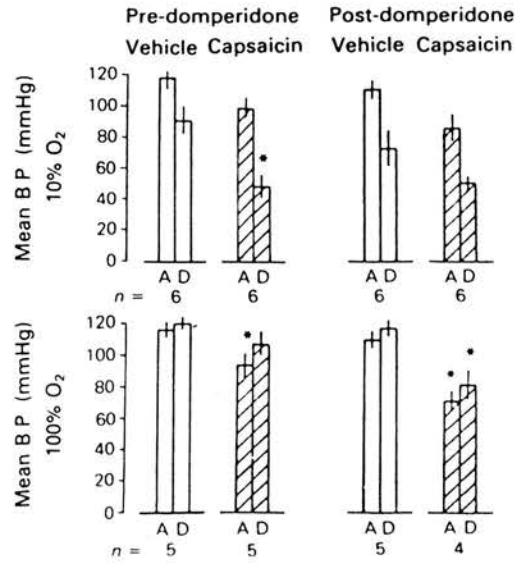

Figure 6 Mean arterial blood pressure of vehicle-treated (open columns) and capsaicin-treated (hatched columns) anaesthetized rats (A) during air breathing and during (D) either 10% O_2 (upper panels – at time of maximum change in BP) or 100% O_2 (lower panels) for 3 min before and after domperidone 100 $\mu g\,kg^{-1}$, i.v. Vertical lines show s.e. mean. $^*P<0.05$ in comparison with corresponding values (pre- and post-domperidone) for vehicle-treated animals using the Wilcoxon two sample test.

related respiratory depressant effect of dopamine was *potentiated* slightly by oxygen, and this effect was antagonized by domperidone. Dompamine-induced reductions in respiration obtained during oxygen breathing were greatly reduced by cutting the carotid sinus nerves, but not by cutting the vagosympathetic trunks in the neck.

Discussion

Our results are not compatible with the hypothesis that the reduced arterial chemosensitivity observed in anaesthetized adult rats which had been treated neonatally with capsaicin is caused by a change in the responsiveness of carotid chemoreceptors to dopamine (see Introduction). Although dopamine levels were five fold higher in the carotid bodies of capsaicin-treated rats in comparison with vehicle-treated controls, there was no significant difference between the two groups in the depression of respiration caused by intravenously injected dopamine, as judged from the ID_{50} values.

The respiratory data confirmed that the respiratory chemoreflex to stimulants such as hypoxia and sodium cyanide is significantly reduced in rats that had been treated neonatally with capsaicin (Bond *et al.*, 1982). Capsaicin mainly destroys unmyelinated sensory afferent fibres when injected neonatally in the doses used in this study (Jancso *et al.*, 1977) and capsaicin-treated rats have fewer unmyelinated fibres in the carotid sinus nerve as compared with vehicle-treated controls (see Bond *et al.*, 1982). Unmyelinated fibres account for 86% of the fibres in the rat sinus nerve (McDonald, 1983), although it is not known how many of these are chemosensory. The question of how destruction of sensory afferents by capsaicin is related to the increase in levels of dopamine, noradrenaline and 5-HT in the carotid body, and increased dopamine levels in the superior cervical ganglia, cannot be answered by the results from our study. It is also likely that other substances will be affected by capsaicin treatment, including substance P (Gamse *et al.*, 1980; Keeler & Black, 1981) which has been found in the rat carotid body (Jacobowitz & Helke, 1980) and might function as a neurotransmitter at the central terminals of afferent carotid sinus nerve fibres (Helke *et al.*, 1980).

The selective dopamine D_2-receptor antagonist domperidone (Baudry *et al.*, 1979; Lazareno & Nahorski, 1982), which is effective in antagonizing the chemodepressant action of dopamine in rabbit carotid bodies (Mir *et al.*, 1984), greatly reduced the hypoventilation caused by dopamine both in capsaicin-treated and vehicle-treated rats, the latter

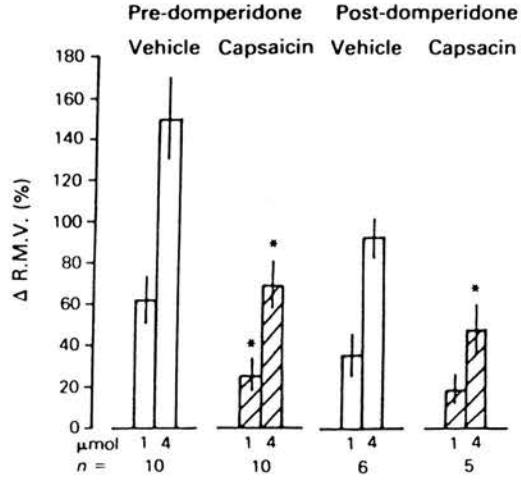

Figure 7 Increases in respiration evoked by two doses of cyanide (1 and 4 μmol i.v.) in *n* vehicle-treated control (open columns) and capsaicin-treated (hatched columns) anaesthetized rats before and after domperidone (100 μg kg^{-1} i.v.). Vertical lines show s.e. mean. Mean basal ventilation was 29.4 ± 2.1 in controls and 34.6 ± 2.9 ml 100 g^{-1} min^{-1} in capsaicin-treated rats before domperidone. Corresponding values post-domperidone were 37.7 ± 5.1 and 41.5 ± 3.5 ml 100 g^{-1} min^{-1}.* $P<0.05$ in comparison with corresponding vehicle-treated controls (Wilcoxon two sample test).

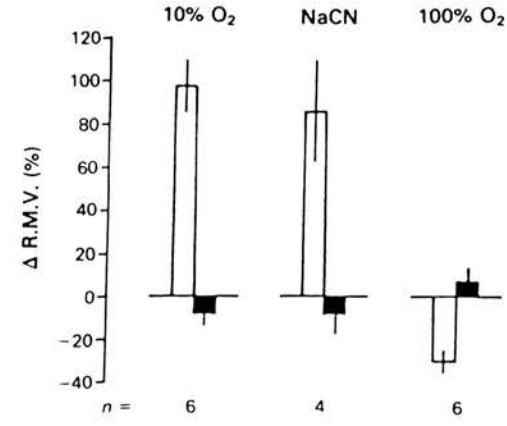

Figure 8 Ventilatory responses to breathing 10% O_2 or 100% O_2, both measured 1 min after switching from air, and to sodium cyanide 1 μmol i.v. during air breathing, were obtained before (open columns) and after (solid columns) cutting both the carotid sinus and vagus nerves in vehicle-treated rats. Vertical lines show s.e. mean. Ventilation in the steady state averaged 25.3 ± 3.1 ml 100 g^{-1} min^{-1} before and 22.9 ± 2.4 ml 100 g^{-1} min^{-1} after sectioning the nerves.

finding confirming the report by Horn *et al.* (1984). This evidence suggests that the respiratory-depressant effect of dopamine is mediated via a dopamine D_2-receptor, and similar conclusions were reached by Zapata *et al.* (1984) on the basis of respiratory experiments in rats using the dopamine antagonists sulpiride and metoclopramide. Since, in general terms, dopamine does not cross the blood brain barrier and neither does domperidone (Laduron & Leysen, 1979), it can be deduced that the respiratory depressant effect of DA must involve actions outside the CNS. The available evidence points to the carotid body as the major site at which dopamine acts to reduce respiration in rats (e.g. Horn *et al.*, 1984; Zapata *et al.*, 1984). Electrical recordings of chemosensory activity in the rat carotid sinus nerve (Cardenas & Zapata, 1981) have shown that, as in most species, dopamine depresses chemosensory discharge and would, therefore, be expected to cause hypoventilation by reducing peripheral chemoreceptor drive to respiration in rats anaesthetized with pentobarbitone (Horn *et al.*, 1984).

Cutting both carotid sinus nerves greatly reduced dopamine-induced ventilatory depression and also attenuated the reflex hyperpnoea caused by hypoxia or cyanide acting on the carotid chemoreceptors, and this evidence is consistent with an inhibitory action of dopamine on carotid body chemoreceptors. About 20% of the responses to hypoxia and cyanide survived bilateral cutting of the sinus nerves, but cutting both vagosympathetic nerves in the neck abolished the residual responses. These extra-carotid effects may have originated from thoracic or abdominal chemoreceptors (see Cardenas & Zapata, 1983) or perhaps from aortic chemoreceptors, although the latter have been found to be non-functional in rats (Sapru & Krieger, 1977a, b; 1978).

There were some differences from a previous study (Bond *et al.*, 1982), mainly in relation to the absolute values for R.M.V. during steady state conditions, and to the arterial blood gas tensions. These differences may, in part, be explained by the fact that in the present experiments the animals were more deeply anaesthetized than was the case previously. This was a deliberate policy in order to make the results more directly comparable with those of Horn *et al.* (1984) and Cardenas & Zapata (1981) who used a 60 mg kg^{-1} dose of pentobarbitone. Unexpected results were obtained when dopamine was injected into vehicle-treated rats which were breathing 100% O_2 under steady-state conditions. It was anticipated that the increased oxygen tension would virtually abolish peripheral chemosensory activity ('physiological denervation' of the chemoreceptors) and so prevent dopamine from depressing ventilation (i.e. there would be no respiratory drive from the chemoreceptors for dopamine to depress). In fact dopamine still caused some hypoventilation, but the responses were not dose-related. Similar, although less intense, effects can be seen in the results of Horn *et al.* (1984). The cause of this responsiveness to dopamine in rats breathing oxygen is difficult to ascertain, but since it was partially antagonized by domperidone it apparently involves actions at dopamine D_2-receptors. Rats anaesthetized with pentobarbitone and breathing oxygen may still have a significant peripheral chemoreceptor drive to respiration either originating from within the carotid body (perhaps involving CO_2) or due to enhanced gain in the CNS, and this possibility could be investigated by recording chemosensory activity from the sinus nerve.

Although capsaicin-treated rats show a reduced respiratory response to peripheral chemoreceptor stimulation, sudden withdrawal of chemosensory input as a consequence of switching to breathing 100% O_2 caused just as marked a hypoventilation in these animals as it did in the vehicle-treated controls. This implies that, although chemoreflex activity is reduced by capsaicin treatment, there is still sufficient chemoreceptor drive to respiration under the conditions of our experiments to give a normal response to oxygen-breathing. Domperidone had no significant effect on oxygen-induced hypoventilation in controls, but did significantly attenuate the response in capsaicin-treated rats. However, it is difficult to evaluate this evidence because systemic arterial blood pressure was significantly lower in capsaicin-treated rats during oxygen breathing after domperidone in comparison with vehicle- treated controls, which complicates the interpretation. Further studies on this aspect are needed.

Ventilatory responses to hypoxia and cyanide were not potentiated after domperidone, yet should have been if endogenous dopamine was tonically inhibiting the chemoreceptors, particularly in capsaicin-treated rats. In fact responses tended to be reduced after the antagonist, although ventilation in the steady state was increased, significantly so in the case of capsaicin-treated rats. Horn *et al.*, (1984) also found a reduced respiratory response to hypoxia following administration of the dopamine D_2-receptor antagonist sulpiride. Thus, domperidone increases ventilation under normoxic conditions in capsaicin-treated rats, which could be taken as evidence for a greater release of dopamine occurring in the carotid bodies of these animals as compared with the vehicle-treated controls. This assumes that exogenous dopamine has similar effects to endogenous, which need not be the case. In contrast, however, the fact that domperidone reduced the ventilatory response to hypoxia could be regarded as evidence for endogenous dopamine, acting via a dopamine D_2-receptor, being involved in chemoexcitation. This is reminiscent of the 'dual effect' described by Car-

denas & Zapata (1980) whereby dopamine reduced chemoreceptor responses to low doses of cyanide but potentiated the effects of higher doses. In our experiments, domperidone may, by blocking the actions of endogenous dopamine, potentiate ventilatory responses when dopamine release is low (i.e. during normoxia) but reduce the responses when release is high (during hypoxia). Whether or not this does occur remains to be established.

In conclusion, within the limitations imposed by working on whole animals where secondary changes can affect primary reflex responses, and the anaesthetic agent provides further complications, it seems unlikely that the increased levels of dopamine found in the carotid bodies of rats treated neonatally with capsaicin are responsible for the reduced peripheral arterial chemoreflex activity because: (a) dopamine-induced ventilatory depression was not significantly altered in capsaicin-treated animals, and (b) the dopamine antagonist domperidone did not increase the respiratory responses evoked by the chemoreceptor stimulants hypoxia and cyanide. Further studies, possibly in conscious animals, are needed to determine how neonatal treatment with the neurotoxin capsaicin causes a reduction in respiratory responsiveness to peripheral chemoreceptor stimulation. It will also be interesting to investigate the extent to which central and/or peripheral effects of capsaicin influence the actions and interactions of polypeptides such as substance P and the enkephalins, which are present in the carotid body and can co-exist with monoamines (Hansen *et al.*, 1982; Varndell *et al.*, 1982).

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SECTION 3

POLYPEPTIDES AND 5-HYDROXYTRYPTAMINE

PAPERS 24 - 35

[*From the Proceedings of the Physiological Society*, 14–15 *July* 1978
Journal of Physiology, **284**, 164–165*P*]

Effects of substance P on carotid chemoreceptor and baroreceptor activity in the cat

By D. S. McQueen. *Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ*

Substance P is a polypeptide which can activate sensory fibres in the rabbit ear (Juan & Lembeck, 1974) and substance P-like material has been detected in peripheral sensory nerve endings (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygårds & Pernow, 1977). It was considered worth performing an electrophysiological investigation to determine whether substance P affects carotid chemoreceptor or baroreceptor activity, particularly since it has been suggested that carotid body Type I cells may secrete a polypeptide (Pearse, 1969).

Cats were anaesthetized with pentobarbitone and electrical activity recorded from the peripheral end of a sectioned sinus nerve. The ganglioglomerular nerves were cut (see McQueen (1977) for details). Low doses of synthetic substance P (1–5 μg into the ipsilateral common carotid artery (I.C.) over 2 sec) caused a small reduction in spontaneous chemosensory activity, while higher doses (10–100 μg I.C.) increased discharge by 1–5 c.p.s. over a 1–2 min period after a delay of 10–15 sec. There was a dose-dependent fall in B.P.

Comparison of chemoreceptor responses obtained before and after administering substance P showed that the excitatory effect of ACh was slightly reduced whereas that of NaCN was slightly potentiated. The inhibitory action of dopamine was unaffected by substance P. When the stimulants were injected I.C. during an infusion of substance P (10 μg/min I.C. for 5 min), responses to ACh were reduced and those to NaCN slightly potentiated. In two cats the effect of substance P (5–10 μg I.C.) on baroreceptor activity was investigated. There was no action on spontaneous activity in the 5–7 sec period following the injection, and thereafter discharge followed the changes in systemic B.P., falling initially, then recovering to control level.

The results show that substance P in the doses studied did not stimulate the carotid baroreceptors and had only a weak effect on chemosensory activity. The slight inhibition of the ACh response could be due to a nicotine-blocking effect of substance P, such as that described for Renshaw cells (Ryall & Belcher, 1977), and weak nicotinic block is known to potentiate the action of NaCN on carotid chemoreceptors (McQueen, 1977). The physiological significance of these results with exogenous substance P depends on whether or not substance P is present in the cat carotid body; Hanbauer (1977) was unable to detect substance P in rat carotid bodies, despite the use of a sensitive radioimmunoassay method.

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EFFECTS OF SUBSTANCE P ON CAROTID CHEMORECEPTOR ACTIVITY IN THE CAT

BY D. S. McQUEEN

From the Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

(*Received 8 May 1979*)

SUMMARY

1. The influence of substance P (SP) on spontaneous chemosensory discharge and on responses of the carotid chemoreceptors to various drugs has been investigated in pentobarbitone anaesthetized cats in which chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve.

2. After an initial slight inhibition during the first 5–15 sec following the injection, SP (0·1–100 μg I.A.) caused a dose-related increase in discharge which lasted for 45–300 sec in artificially ventilated cats, discharge being increased by about 50% on average. The increase was of shorter duration when the animals were allowed to breathe spontaneously.

3. The delayed increase in discharge was not secondary to the hypotension caused by SP, nor was it entirely due to changes in bronchomotor tone resulting from direct or indirect actions of SP, although such changes contributed to the response. It was not possible to determine whether the excitation was due to a direct effect of SP on the chemoreceptors.

4. Chemosensory excitation evoked by NaCN (5 μg I.A.) was potentiated during I.A. infusions of SP and also 10–20 min after SP (10 μg I.A.) had been injected. In contrast, responses to ACh (50 μg I.A.) were inhibited. These effects may be due to a nicotinic-blocking action of SP on the carotid chemoreceptors. It was also found that the inhibitory action of dopamine (5 μg I.A.) was reduced during SP infusion whereas that of 5-HT (10 μg I.A.) was potentiated.

5. A sample of crude SP had effects on spontaneous chemoreceptor discharge and responses to NaCN and ACh which were qualitatively similar to those obtained using synthetic SP.

6. The physiological significance of the results is discussed and it is concluded that the interpretation depends upon whether or not SP is present in the cat's carotid body.

INTRODUCTION

The carotid body type 1 cell is considered by Pearse (1969) to be a member of the APUD cell series (i.e. cells which share the characteristics of *a*mine and amine *p*recursor *u*ptake and *d*ecarboxylation, and polypeptide secretion). Pearse suggested that the type 1 cell secretes a low molecular weight polypeptide, which he tentatively named 'glomin'. Histological evidence has since been obtained which indicates

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that polypeptide or protein-containing granules are present in mammalian carotid body cells (Capella & Solcia, 1971; Pearse, Polak, Rost, Fontaine, Le Lièvre & Le Douarin, 1973).

Immunohistochemical evidence (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygåards & Pernow, 1977; Cuello, 1978) shows that SP-like material is present in the peripheral endings of various sensory nerves and it has also been reported that synthetic SP can stimulate sensory nerve endings (Juan & Lembeck, 1974).

As there is a possibility that carotid body cells secrete a polypeptide, and it is known that SP-like material is associated with some peripheral sensory nerve endings, it seemed worth determining what effect SP has on the cat carotid chemoreceptors. A preliminary report on some of the results has been made to the Physiological Society (McQueen, 1978*a*).

METHODS

Most of the details have been described previously (McQueen, 1977; Docherty & McQueen, 1978) and only a brief summary follows. Experiments were performed on cats of either sex weighing between 2·2 and 3·7 kg (median weight 2·8 kg, $n = 11$) which were anaesthetized with pentobarbitone sodium (42 mg/kg I.P.) supplemented approximately every 1·5–2 hr during the experiment by I.V. administration of 10 % of the initial dose. Blood pressure was recorded from one femoral artery and the other femoral artery was cannulated for arterial blood sampling. Rectal temperature was maintained at 38 ± 0·5 °C and the bladder drained at regular intervals.

A sinus nerve was dissected free from surrounding tissues, cut centrally, and the electrical activity of single or multiple chemoreceptor units (two to four active units preferred) recorded from the peripheral nerve using bipolar platinum electrodes and an a.c. amplifier (Neurolog; Digitimer). The ganglio-glomerular nerves were cut in order to prevent changes in sympathetic nerve activity influencing chemoreceptor discharge (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961*a*). For part of some experiments the animals breathed spontaneously and respiration was recorded via an integrating pneumotachograph (CS3c; Mercury Electronics) which was used in conjunction with a time clock (2112; Palmer) to provide a cumulative record of total volume inspired over a period of 30 sec. A 'staircase' tracing was obtained on the pen recorder (MX6; Lectromed) giving a breath-by-breath record of respiration with the over-all height being proportional to the respiratory half-minute volume. For the rest of the experiments the animals were artificially ventilated with room air and usually paralysed by gallamine triethiodide (3 mg/kg I.V.). End-tidal CO_2 was continuously monitored by an infrared CO_2 analyser (Med 1A; Grubb Parsons) and the $P_{a.co_2}$, $P_{a.o_2}$ and pH of femoral arterial blood samples measured using a gas monitor (BMS3 with PHM71 meter, Radiometer).

Nerve activity was recorded on magnetic tape (Tandberg 100; frequency response d.c. to 1250 Hz) and subsequently analysed with the aid of a computer (PDP-8; Digital Equipment Corporation) in order to provide data concerning discharge frequency. The average ($\bar{x}$ ct/sec) and total count (Σx) were calculated for each response after its duration (t sec) had been determined. Responses were expressed as a change from control level by subtracting the appropriate control or background discharge (i.e. as $\Delta\Sigma x$). Data from different experiments were pooled and the results presented as the mean ± S.E. of mean.

Drug solutions (0·1 ml.) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded and washed in with 0·2 ml. modified Locke solution which had been bubbled with 5 % CO_2 in air in a water bath at 37 °C; the wash solution had no effect on spontaneous chemoreceptor discharge. The catheter was introduced into the common carotid artery via the lingual artery, and its tip lay about 2 cm caudal to the carotid bifurcation. Injections were made over a 2 sec period and at least 20 min allowed to elapse between doses of SP. Drug *infusions* were made through a second catheter situated in the common carotid artery at its junction with the superior thyroid artery (through which the catheter was introduced) using an infusion pump (Braun) which delivered 0·5 ml./min of drug

solution. Injections of stimulants NaCN or acetylcholine (ACh)) into either of the two carotid catheters evoked very similar chemoreceptor responses.

Drugs were prepared in modified Locke solution (NaCl 6·0 g; KCl 0·42; $CaCl_2$ 0·24; Tris base 6·0 g; *N*-HCl, 39 ml.; distilled water to 1 l.; pH 7·41 at 37 °C). Doses referred to are those of the salts. Glassware used for SP was silanized. The drugs used in this study were: pentobarbitone sodium, gallamine triethiodide (May & Baker); acetylcholine iodide, atropine sulphate, sodium cyanide (B.D.H.); dopamine hydrochloride (Koch-Light); 5-hydroxytryptamine creatinine sulphate, synthetic Substance P (Sigma; Beckman).

A sample of SP which had belonged to Professor Gaddum was kindly given to me by Dr T. B. B. Crawford of this department. The 1 mg sample of crude SP had been extracted from horse intestine, adsorbed onto lactose and stored in a nitrogen-filled sealed ampoule. Its activity was 75 units/mg, which is approximately equivalent to 0·38 μg synthetic SP/mg extract (Euler, 1977).

RESULTS

Injection of SP

In artificially ventilated cats SP injected close-arterial to the carotid body in a dose of 0·1–100 μg usually caused a slight inhibition of spontaneous chemoreceptor discharge during the first 5–15 sec after the injection, an effect which did not appear to be dose-related. The initial inhibition was followed by an increase in discharge which, although not substantial, lasted for 45–300 sec and was dose-related (see Figs. 1 and 2). Low doses of SP (0·1–2·5 μg) did not always cause a delayed increase in discharge: there was often no change. Tachyphylaxis seemed to develop to the delayed increase in chemoreceptor discharge, although not to the hypotension which also followed the injection of SP, and it was noted that the biggest increases in discharge were obtained in female cats.

Data obtained from five experiments in which chemoreceptor discharge, expressed as a percentage of the control spontaneous frequency, was pooled and plotted against time after an I.A. injection of SP are shown in Fig. 2. This quantitative evidence confirmed that higher doses of SP caused a delayed increase in chemoreceptor discharge, albeit a slight and somewhat variable effect. The duration of the increase, but not necessarily the peak discharge, appeared to be dose-related.

SP also caused a fall in arterial blood pressure which lasted for 1–5 min and was usually, but not invariably, polyphasic. The duration of the hypotension (i.e. time taken to return to control B.P.) was generally dose-related although the magnitude of the peak fall in pressure was not always related to the dose of SP (see Fig. 1). The increase in chemoreceptor discharge associated with higher doses of SP coincided with the onset of hypotension. However, although low doses of SP often caused B.P. falls similar to those evoked by much higher doses (see also Vogler, Haefely, Hürlimann, Studer, Lergier, Strässle & Berneis, 1963), there was often very little or no increase in discharge, and sometimes an increase in discharge occurred which was accompanied by a *rise* in B.P. (Figs. 3 and 4). These results suggested that the increase in chemoreceptor activity was not necessarily secondary to the hypotension caused by SP, and this was confirmed by showing that chemoreceptor discharge still increased when the fall in B.P. was prevented by infusing dextran after the injection of SP (Fig. 4), or in cases where mean B.P. rose rather than fell.

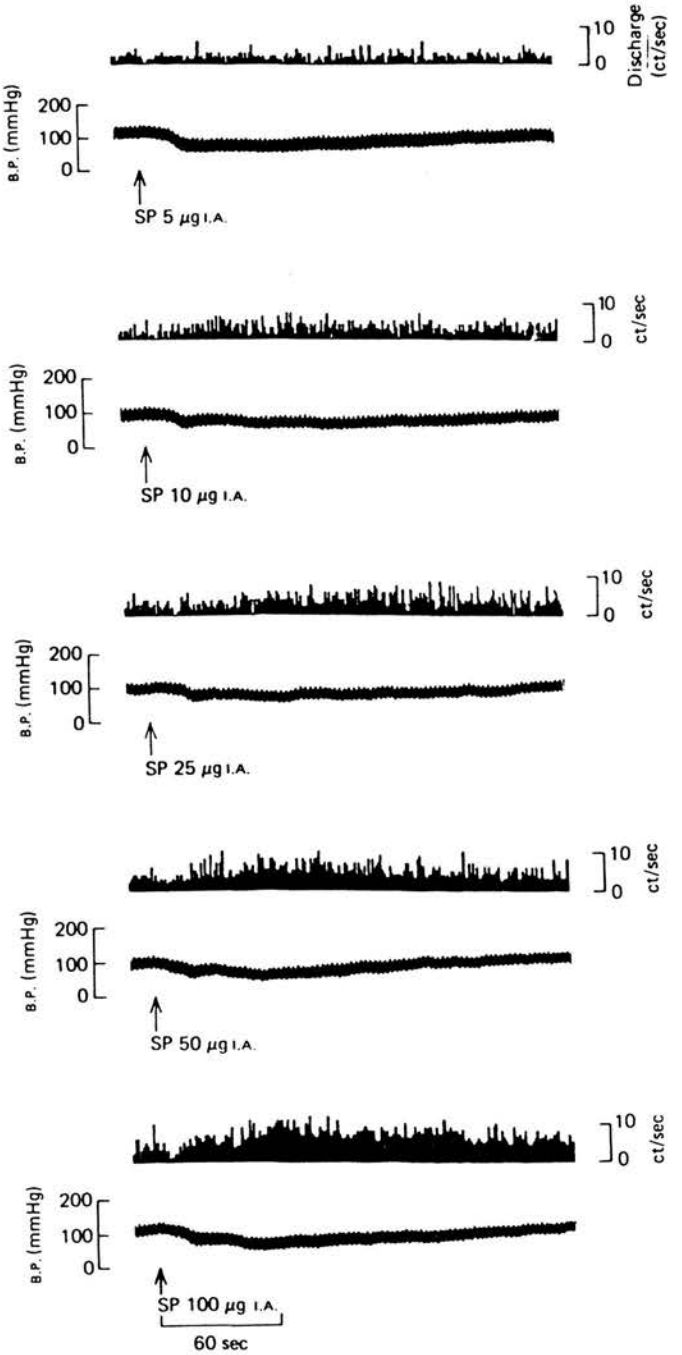

Fig. 1. Response of a single chemoreceptor unit to various doses of SP injected I.A. in random order with at least 20 min between successive doses. Each panel shows a block diagram of the chemoreceptor discharge in ct/sec and femoral arterial blood pressure. It can be seen in this particular experiment that although a similar fall in B.P. was evoked by all the doses of SP, the delayed increase in chemoreceptor discharge was dose-dependent.

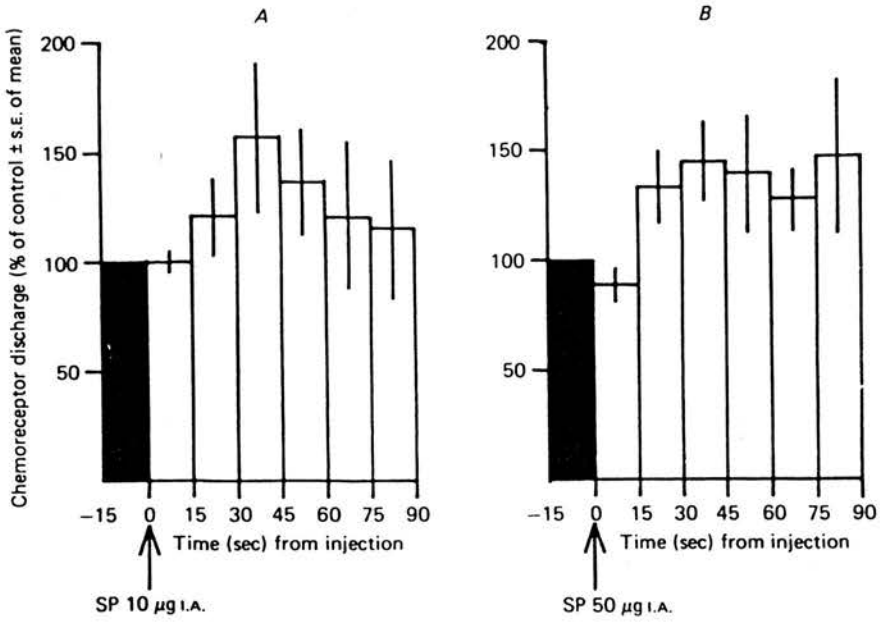

Fig. 2. Block diagrams showing chemoreceptor discharge following I.A. injections of 10 μg SP (*A*) and 50 μg SP (*B*). Discharge was averaged over 15 sec periods and expressed as a percentage of the average discharge in the 15 sec control period before the injection (black rectangle). Data obtained in different experiments were pooled and are shown as the mean percentage ± S.E. of the mean. The over-all average discharge in the control period was 2·8 ± 0·9 ct/sec for the four experiments with SP 10 μg I.A., and 2·5 ± 0·8 ct/sec for the five experiments with SP 50 μg I.A.

Bronchial and respiratory changes

In some experiments end-tidal CO_2 increased slightly at the time when spontaneous chemoreceptor discharge increased following SP I.A. This was reminiscent of the delayed (bigger) increase in end-tidal CO_2 seen following I.A. methacholine, an effect which was attributed to bronchoconstriction (McQueen, 1978*b*). It was necessary, therefore, to investigate whether the delayed increase in chemoreceptor discharge observed in the present experiments was secondary to changes in blood gas tensions resulting from a bronchoconstrictor action of SP; it is known that SP can cause bronchoconstriction in guinea-pig lung (Bisset & Lewis, 1962; Bhoola, Collier, Schachter & Shorley, 1962).

If bronchoconstriction were responsible for the delayed increase in discharge, one would not expect to see the effect in a spontaneously breathing animal because as soon as gas tensions began to change as a result of bronchoconstriction, the central and remaining peripheral chemoreceptors would reflexly adjust ventilation. Recordings of chemoreceptor activity were obtained in three experiments and responses to SP obtained with the animals breathing spontaneously and again when they were artificially ventilated and paralysed. The results obtained are illustrated in Figs. 3 and 4. It was found that the increase in chemoreceptor discharge occurred during spontaneous respiration, although the effect was not so sustained

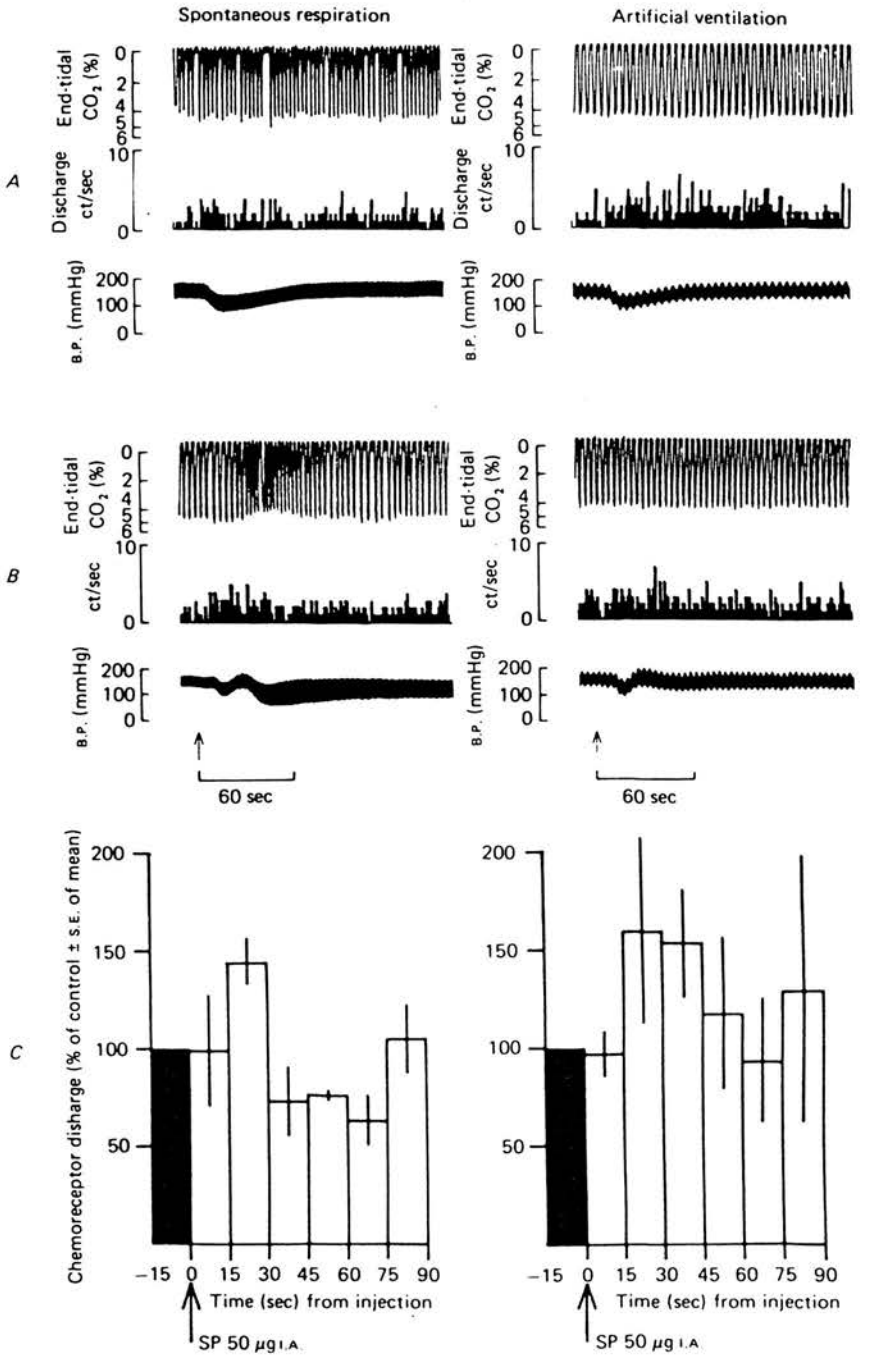

Fig. 3. Chemoreceptor discharge evoked by SP during spontaneous respiration compared with the effect observed on the same recording under conditions of artificial ventilation. *A* is the chemoreceptor discharge (three unit recording) in response to SP 1 μg I.A. *B* is from a different animal (two unit recording) showing the effects of SP 50 μg I.A. *C* presents the pooled data from three experiments in which SP 50 μg I.A. was injected at time 0, block diagram details being the same as for Fig. 2. The average control discharge was 1·3 ± 0·4 ct/sec during spontaneous breathing and 2·0 ± 0·8 ct/sec during artificial ventilation. The panels in *A* and *B* show, from above downwards. end-tidal CO_2; block diagram of chemoreceptor discharge in ct/sec; femoral arterial B.P. Injections represented by the arrows.

as that seen following the I.A. injection of SP when the animals were artificially ventilated (see Figs. 3 and 4). Arterial blood samples taken 45 sec after the injection of SP showed a fall in P_{a,O_2} and a rise in P_{a,CO_2} in artificially ventilated cats, but increases in P_{a,O_2} and slight decreases in P_{a,CO_2}, or no changes at all, when the animals were breathing spontaneously (see Fig. 4). The changes in gas tensions seen under conditions of artificial respiration were only slight (1–3 torr increase in P_{a,CO_2}; 1–5 torr decrease in P_{a,O_2}) but, because they interact (see Fig. 3 in Eyzaguirre & Lewin, 1961*b*), are probably responsible for some of the more *delayed* increases in chemoreceptor discharge.

Increases in chemoreceptor discharge were obtained when the animals were breathing spontaneously, despite the increase in respiration which occurred, even after the lowest dose of SP studied (0·1 μg I.A.). When the hypotensive action of SP was prevented by infusing dextran I.V. (see Fig. 4), there was no longer any marked change in respiration, thereby indicating that the increase in respiration was secondary to the hypotension.

Influence of SP injections on responses to ACh, NaCN, dopamine and hypoxia

During the study of the effects of SP on chemoreceptor discharge, responses to ACh (50 μg I.A.) and NaCN (5 μg I.A.) were determined before and 10–20 min after injecting SP in three experiments on artificially ventilated paralysed cats. Results obtained are shown in Fig. 5*A* and it was found that responses to NaCN were potentiated following SP, whereas those to ACh were slightly reduced. Inhibition evoked by dopamine (5 μg I.A.) was unaltered after SP. In two experiments SP (10 μg I.A.) was injected at the peak of the response to hypoxia (animal breathing 5% O_2/95% N_2) and had no effect on discharge. Although the results showed a potentiation of the response to NaCN, they were difficult to interpret because it was not known whether SP was directly influencing the chemoreceptors 10–20 min after it had been injected.

Infusions of SP

It was suspected that tachyphylaxis develops to the effects of SP on the chemoreceptors, so only short infusions of SP were studied in artificially ventilated paralysed cats. Since it was impossible to obtain dose–response data from such short infusions, single submaximal doses of chemoreceptor stimulants were used and responses obtained before and during a 5 min infusion of SP (1–50 μg/min) into one of the carotid catheters (see Methods). Sodium cyanide was injected into the other catheter 2 min after the infusion began, and ACh injected 4·5 min into the infusion. A fall in B.P. occurred and was usually sustained for most of the infusion period.

Results from three experiments are summarized in Fig. 5*C*. There was a dose-dependent potentiation of responses to NaCN and an inhibition of responses to ACh during the infusion of higher doses of SP. Responses to ACh and NaCN were largely unaltered when the drugs were injected during an infusion of 1 μg SP/min, or during the infusion of Locke solution. Spontaneous discharge was affected variably during the SP infusions, there being no obvious trend (see Fig. 5).

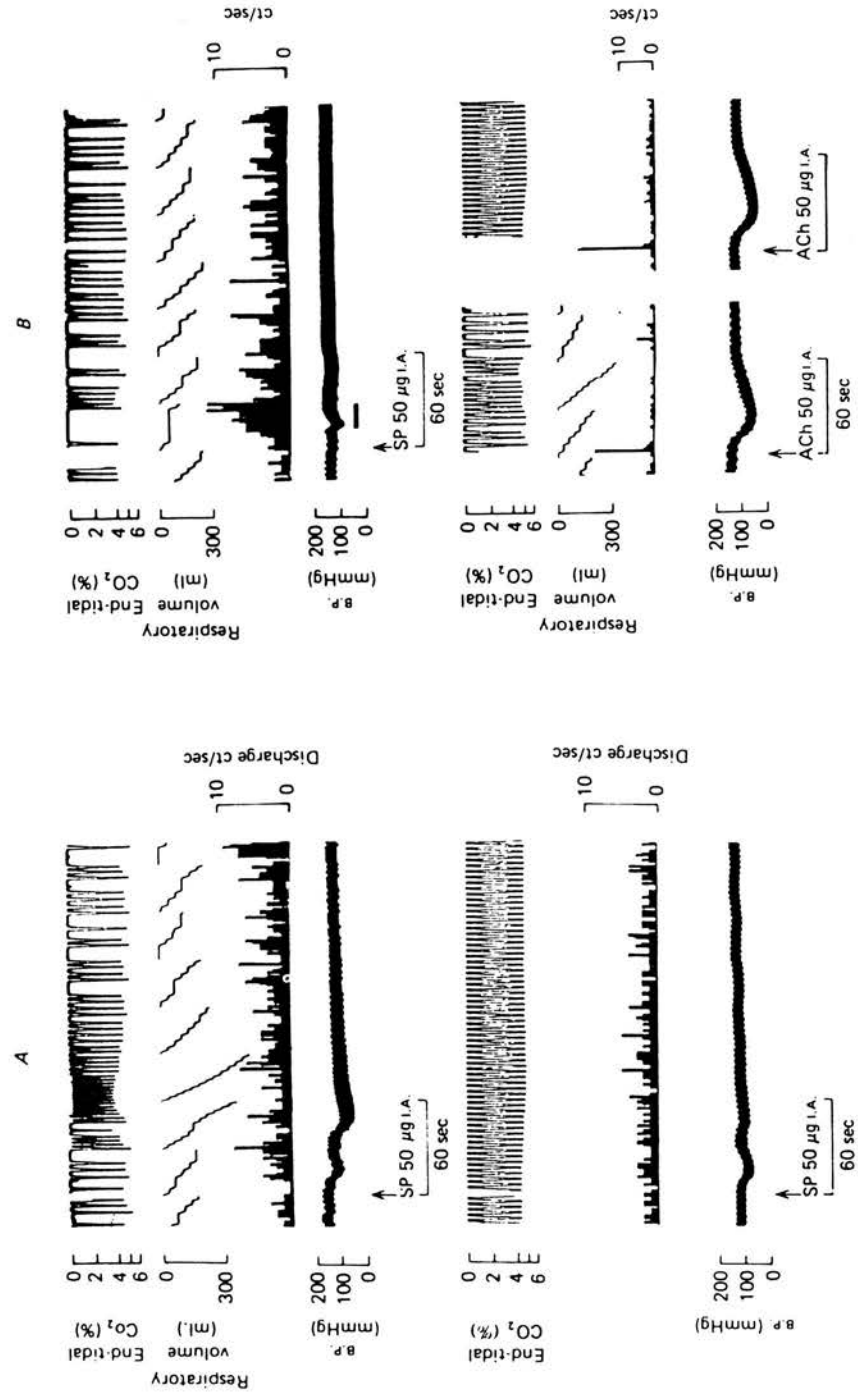

Fig. 4. Responses obtained during an experiment showing the effects on chemoreceptor discharge (three unit recording) of: *A*, SP 50 μg I.A. with the animal breathing spontaneously; *B*, SP 50 μg I.A. with the animal breathing spontaneously and dextran solution infused I.V. during the period represented by the horizontal black bar to maintain mean B.P.; *C*, SP 50 μg I.A. with the animal artificially ventilated. Responses to ACh 50 μg I.A. were also obtained during spontaneous breathing (*D*) and artificial ventilation (*E*).

Panels show from above downwards: end-tidal CO_2, the trace being interrupted at certain points because of the need to record voice on the same channel of the tape recorder; breath-by-breath record of respiration, total height representing the respiratory volume in 30 sec (only shown when the animal was breathing spontaneously); block diagram of chemoreceptor discharge in ct/sec; femoral B.P. Arterial blood samples were taken just before and also 45 sec after ⌒ SP injections. Readings ($P_{a.CO_2}$, $P_{a.O_2}$ mm Hg) were ⌒33, 87 before, and 30, 100 after SP; *B*: 30, 97 before, and 30, 94 after SP; *C*: 31, 105 before, and 33, 100 after SP 50 μg I.A.

Shorter infusions of SP

These experiments were performed in order to avoid some of the problems associated with longer infusions (e.g. the risk of tachyphylaxis; sustained changes in B.P. or blood gas tensions leading to alterations in chemoreceptor sensitivity). Responses to ACh, NaCN, dopamine, and 5-HT were examined individually during short (60 sec) I.A. infusions of SP, the injection being made 45 sec after the start of the SP infusion. The order in which the different concentrations of SP were infused was varied randomly from experiment to experiment, and infusion of the same volume of Locke solution (0·5 ml. over 1 min) had no effect on the responses.

Results obtained from three experiments are summarized in Fig. 5*B*. During these short infusions there was a dose-related increase in responses evoked by NaCN, an effect which although less intense, is similar to that seen during the longer infusions. Responses to ACh were reduced, slightly during infusion of 1 μgSP/min and more markedly during 50 μg/min, this being similar to the results obtained during the longer infusions. However, responses to ACh obtained during 10 μgSP/min, although somewhat variable, were potentiated, whereas they were inhibited during the longer infusion.

The inhibiting response evoked by dopamine (5 μg I.A.) was reduced during SP 1 μg/min (to 52 ± 19 % of the pre-infusion or control response, $n = 3$; background (control) = $3{\cdot}6 \pm 2{\cdot}9$ ct/sec; during infusion = $1{\cdot}1 \pm 0{\cdot}7$ ct/sec) and SP 50 μg/min (to 64 ± 5 %, $n = 2$; background (control) = $1{\cdot}1 \pm 0{\cdot}5$, during infusion = $0{\cdot}4 \pm 0{\cdot}3$ ct/sec). During SP 10 μg/min there was a very variable effect with, over-all, a reduction of the inhibition (to 92 ± 26 %, $n = 3$; background (control) = $1{\cdot}2 \pm 0{\cdot}7$, during infusion $1{\cdot}0 \pm 0{\cdot}5$ ct/sec). The trend was, therefore, a reduction in the inhibitory response to dopamine during SP infusions, an effect which was not obviously dose-related. It should be noted that background activity decreased slightly during the SP infusions.

The brief stimulation of chemoreceptor activity evoked by 5-HT (10 μg I.A.) was reduced during SP infusions of 1 and 10 μg/min in one experiment. The delayed inhibition which followed the initial excitation (see Docherty & McQueen, 1978) was, however, potentiated during both the 1 and 10 μg/min infusions, although background discharge was slightly decreased by the SP.

Spontaneous chemoreceptor discharge was affected variably during the infusions of SP, there being a slight tendency for it to be decreased (see Fig. 5 and the dopamine results).

Injection and infusion of crude SP

A sample of Gaddum's SP was *injected* I.A. in an artificially ventilated cat which had not been paralysed. A slight fall in B.P. occurred and was followed by a rise in pressure. There was no immediate change in spontaneous chemoreceptor discharge, but after a delay of about 30 sec there was an increase which lasted for about 90 sec (see Fig. 6). Injection of the same amount of SP I.V. caused a slight fall in B.P. and also evoked a slight delayed increase in chemoreceptor discharge lasting from 40–120 sec after the injection.

During the *infusion* of crude SP (20 μg/min I.A. for 6 min) the response to NaCN

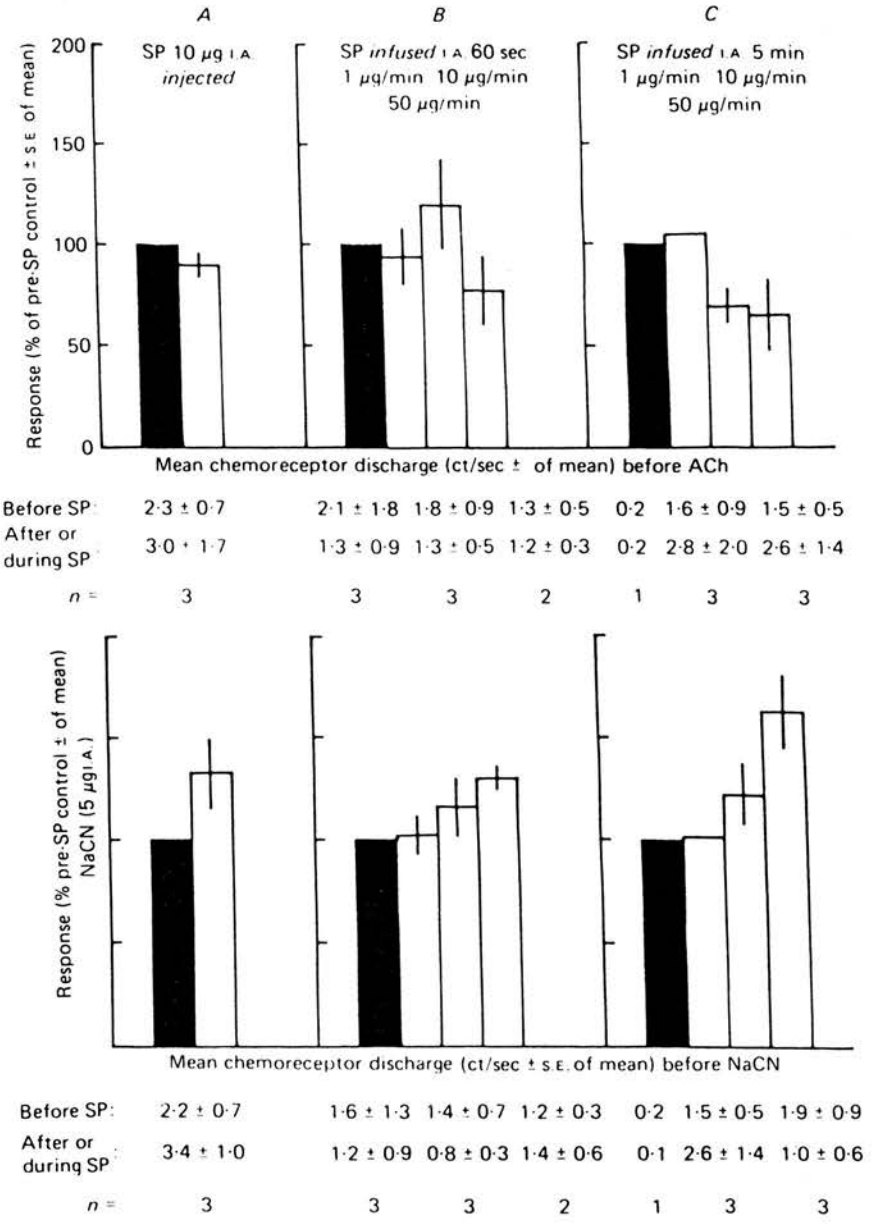

Fig. 5. Pooled data, obtained from the number of recordings indicated, showing the influence of SP on the response of the chemoreceptors to ACh 50 μg I.A. and NaCN 5 μg I.A. Responses are expressed as a percentage of the response ($\Delta\Sigma x$) obtained before SP was administered (black rectangle = control = 100 %). *A* shows the responses to ACh and NaCN injected 10–15 min after a single *injection* of SP (10 μg I.A.). *B* shows the effect on the responses evoked by the stimulants of a 60 sec *infusion* of SP at 1, 10 or 50 μg/min, the ACh or NaCN being injected 45 sec after the start of the infusion. *C* shows the responses evoked during a 5 min *infusion* of SP, NaCN being injected 2 min after the infusion started and ACh 4·5 min into the infusion. The average chemoreceptor discharge in the control periods is shown.

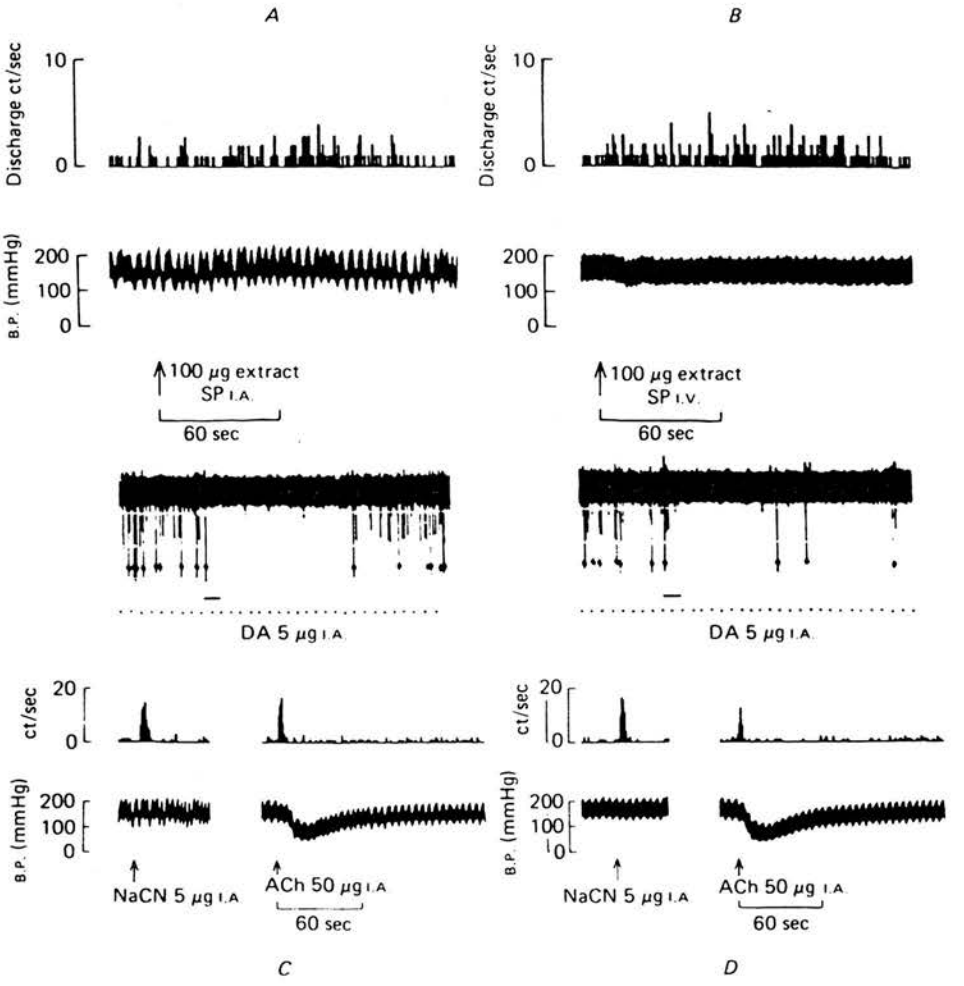

Fig. 6. Chemoreceptor activity (two units) from an experiment in which an extract of SP (1 mg extract ≙ 0·38 μg synthetic SP) was investigated. In *A* 100 μg extract was injected I.A. at the time indicated by the arrow, whereas in *B* the same dose was administered I.V. The animal was artificially ventilated but not paralysed. *C* shows responses to dopamine (DA) 5 μg, NaCN 5 μg and ACh 50 μg obtained during an infusion of Locke solution, whereas those in *D* are the responses obtained during a 6 min infusion of SP (20 μg extract/min). The neurograms show the action potentials with 1 sec timing marks below them, dopamine injections being represented by the horizontal bar. Action potentials were counted at the level represented by the brightening pulse. In the other panels, the upper trace is a bar diagram of chemoreceptor discharge (in ct/sec) with the lower trace showing B.P.

(injected at 3·5 min) was essentially similar to that obtained pre- and post-infusion (see Fig. 6). The response to ACh (injected at 5·5 min) was, however, reduced by about 30 % ($\Delta\Sigma x$) during the SP infusion, and there was a prolonged inhibition of spontaneous discharge following the initial excitation, an effect not observed before or after the infusion. The inhibitory action of dopamine (5 μg injected 1·5 min after the infusion started) also seemed to be potentiated during the infusion of crude SP.

DISCUSSION

Effect of SP on spontaneous chemoreceptor discharge

There was no evidence that exogenous SP caused any substantial change in spontaneous chemoreceptor discharge, such as is seen during the first 10 sec following an I.A. injection of ACh, NaCN or dopamine. However, about 10–20 sec after SP had been injected a long-lasting dose-dependent increase in discharge occurred. There were no consistent changes in spontaneous discharge during *infusions* of SP (1–50 μg/min I.A.), so it appears that the delayed increase is only obtained following the *injection* of high doses of SP. A classical extract of SP caused a delayed increase in discharge qualitatively similar to that evoked by synthetic SP.

Vascular effects. SP caused hypotension followed by a slight rise in B.P. (see also Pernow, 1953), and the present results confirmed that although the duration of the hypotension is dose-related (Vogler *et al.* 1963), it is often difficult to establish a clear dose–response relationship for the effect in cats (Burcher, Atterhög, Pernow & Rosell, 1977). The delayed onset of the increase in chemoreceptor discharge caused by SP and the fact that it tended to coincide with the fall in B.P. raised the question whether it was secondary to the hypotension. The sympathetic nerve supply to the carotid body had been cut, thereby eliminating the possibility that reflex changes in sympathetic activity, secondary to the hypotension, were responsible for increasing discharge by reducing blood flow through the glomus (Floyd & Neil, 1952; Biscoe & Purves, 1967). Chemoreceptor discharge is largely independent of B.P. over the physiological range (Hornbein, Griffo & Roos, 1961; Biscoe, Purves & Sampson, 1970; Acker, Keller, Lübbers, Bingham, Schulze & Caspers, 1973), as is demonstrated in the present experiments by the observation that although ACh, like SP, causes a fall in B.P., there was no marked increase in discharge associated with the hypotension (see Figs. 4 and 6).

The results showed that there was no correlation between the fall in B.P. and the delayed increase in discharge. In spontaneously breathing cats hypotension increased ventilation, an effect which is probably a consequence of several factors including changes in blood flow to the C.N.S. (Schmidt, 1928) and alterations in baroreceptor influences on bronchomotor tone (Daly & Schweitzer, 1951; Heymans & Neil, 1958; Widdicombe, 1963). Hyperventilation would tend to mask any delayed excitatory action of SP on the chemoreceptors. Although it is conceivable that SP could be influencing chemoreceptor discharge by altering the distribution of blood within the carotid body, the balance of evidence makes it unlikely that the delayed increase in chemoreceptor discharge is secondary to vascular effects of SP.

Bronchial effects. Evidence obtained from experiments on artificially ventilated cats showed that injected SP causes delayed changes in arterial blood gas tensions. This effect might have been the result of SP acting directly or indirectly in the lung to cause bronchoconstriction, although whether this was in fact the mechanism remains to be established. However, the increase in chemoreceptor activity, although not so sustained, still occurred in spontaneously breathing cats, even though the arterial blood gas tensions were either unaltered or changed in the opposite direction (P_{a,O_2} increased, P_{a,CO_2} decreased) as a consequence of hyperventilation secondary to the hypotension which followed the SP injection (see above). It appears, therefore,

that the increase in chemoreceptor discharge seen following an I.A. injection of SP can occur independently of respiratory or bronchial changes caused by SP, although such changes do modify the magnitude and duration of the increase. Since vascular and bronchial changes caused by SP are unable to account entirely for the increase in chemoreceptor discharge, particularly during the early phase of the response, and since some other potential secondary explanations can be precluded (e.g. SP does not release catecholamines from the cat adrenal medulla (Feldberg & Lewis, 1964; Lewis & Reit, 1966)), the effect could be due to a direct action of SP on the chemoreceptors.

Direct action of SP on chemoreceptors. It is fairly well established that SP can excite certain neurones in the C.N.S. (Otsuka, Konishi & Takahashi, 1975) and it has been hypothesized that SP may have a physiological role as a central neurotransmitter (Lembeck, 1953) or modulator of neural activity (Krivoy, Kroeger & Zimmermann, 1977). As far as the peripheral nervous system is concerned, Juan & Lembeck (1974) reported that synthetic SP excited peripheral sensory nerve endings associated with paravascular pain receptors in the rabbit ear. They noted a delay of '4–10 sec or more' before neural activity increased in their preparation and Krnjević (1977) also found a delay of 10–30 sec before an excitatory effect was observed following the ionophoretic application of SP to central neurones in cats. The latency and long time course of the effect has been attributed to the time taken for SP to diffuse through neural tissue (Otsuka & Konishi, 1977). However, the possibility that SP may be releasing another agent, or metabolized to an active entity, cannot be precluded. In the present experiments it was not possible to determine whether the chemoreceptors were uniformly affected by SP, or whether there was a difference between, for example, units associated with A fibres and those with C fibres. More recent evidence casts doubts on whether SP can, in fact, stimulate sensory nerve endings. Lembeck, Gamse & Juan (1977) found that synthetic SP was devoid of effect on paravascular pain receptors and were unable to explain the increase in activity obtained with synthetic SP by Juan & Lembeck (1974).

It is possible that SP was acting directly on the chemoreceptors to cause the increase in discharge. Any early excitatory action may have been masked by vascular effects of SP within the carotid body, or by an inhibitory action of SP, directly or indirectly mediated, on the chemoreceptors. However, whether the chemoreceptor-stimulating effect observed in the present experiments is due to primary or secondary actions of SP can only be resolved by further studies using a preparation, such as the *in vitro* carotid body (Eyzaguirre & Lewin, 1961*c*), which avoids many of the secondary complications.

Influence of SP on chemoreceptor responses to ACh, NaCN and dopamine

The results showed that the stimulant action of ACh was reduced after injections of SP and also during infusions of SP, with the exception of the unexplained potentiation during the short 10 μg/min infusion. In contrast, the excitant effect of NaCN was potentiated after injections of SP and, in a dose-related manner, during infusions of SP. Whatever effect SP was exerting on the chemoreceptors was evidently dose-dependent and fairly long-lasting: the response to NaCN was potentiated

10–15 min after the injection of SP (10 μg I.A.). Responses during a prolonged infusion of SP were slightly greater than those obtained during a short infusion, which provides evidence that tachyphylaxis to SP did not occur and also that changes in responsiveness during prolonged infusions were not secondary to sustained changes in B.P. or blood gas tensions evoked by SP.

SP has a nicotinic-blocking action, as has been shown by Ryall & Belcher (1977) on Renshaw cells and by Livett, Kozousek, Mizobe & Dean (1979) on cultured adrenal chromaffin cells. The present results are compatible with a slight nicotinic-blocking action of SP on the carotid chemoreceptors because such an effect would reduce the response to ACh and potentiate the action of cyanide (McQueen, 1977). Further experiments are required to determine whether this is, in fact, the mechanism of action of SP on the chemoreceptors.

The present results confirm previous reports that dopamine inhibits spontaneous chemosensory discharge as does 5-HT, after a brief initial excitation (e.g. see Docherty & McQueen, 1978). The inhibition evoked by dopamine was reduced during SP infusions whereas that associated with 5-HT was potentiated. It was not possible to determine what was responsible for these effects, nor what effect SP was having on the release of dopamine or 5-HT, both of which are present in the carotid body (Chiocchio, Biscardi & Tramezzani, 1967; Chiocchio, King, Carballo & Angelacos, 1971). It has been shown in cats that intra-nigral SP results in an increased release of dopamine (Chéramy, Niedullon, Michelot & Glowinski, 1977) and, in rats, that SP injected into the lateral ventricles stimulates the synthesis and utilization of dopamine and 5-HT in brain (Carlsson, Magnusson, Fisher, Chang & Folkers, 1977). SP has also been shown to be capable of affecting the release of 5-HT in the rat substantia nigra (Reubi, Emson, Jessell & Iversen, 1978). It would seem worth investigating whether SP influences carotid body dopamine and 5-HT; the outcome of such studies might explain the present results with exogenous dopamine and 5-HT.

Physiological significance of the results

The finding that spontaneous chemoreceptor discharge is slightly increased following close-arterial injection of SP to the carotid body, and that SP may be causing a slight nicotinic-blocking action on the chemoreceptors, might be considered to be of pharmacological rather than physiological interest because high doses of SP were needed to show the effects. It has also to be borne in mind that exogenous synthetic SP may not be mimicking the effect of any endogenous SP-like material that may be present in the carotid body; vascular or other non-specific effects of the injected SP may mask the primary action, and effects on cell metabolism (e.g. affecting dopamine or 5-HT as discussed above), glomus blood vessels, or even as a hormone, may be of more importance than any direct effect on sensory nerve activity. In any event, the present experiments would only detect direct effects of exogenous SP on the chemosensory mechanism which show up within a few minutes, which may not be the appropriate time course for any physiological action. A specific SP antagonist would have been very useful in the present experiments, but unfortunately none exists; baclofen (Saito, Konishi & Otsuka, 1975) is not a specific SP antagonist (Carlsson *et al.* 1977; Krnjević, 1977).

The physiological significance of the results depends on whether or not SP is present in the cat carotid body. As mentioned in the Introduction, there is evidence that a polypeptide is present in the carotid body, and SP-like material has been detected in various peripheral sensory nerves; SP is probably released from nerve endings of small C fibres (Hökfelt *et al.* 1977). Recent immunohistochemical evidence shows that SP-like material is present in some 5-HT-containing neurones in the rat C.N.S. (Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow & Goldstein, 1978) and, as mentioned above, there are large numbers of 5-HT-containing cells in the cat carotid body. But is there any SP in the carotid body? Hanbauer (1977) was unable to detect any in the rat carotid body using a sensitive radioimmunoassay technique, but whether or not there is any in the cat carotid body remains to be established.

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SUBSTANCE P: A CAROTID BODY PEPTIDE

A.C. CUELLO and D.S. McQUEEN

Departments of Pharmacology & Human Anatomy, University of Oxford, Oxford OX1 3QT and Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ (United Kingdom)

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SUMMARY

Immunofluorescent studies have shown that substance P or substance P-like material is present in the cat carotid body, being located in some of the glomus cells and also in nerve axons. About 20% of the glomus cells were substance P positive. Substance P may be stored in glomus cells containing monoamine(s), but further studies are needed to investigate this aspect and also to determine what role, if any, substance P plays in the process of chemoreception.

Pearse [13] proposed that the carotid body type 1 cells, also known as glomus, chief, or chemoreceptor cells [1], secrete a low molecular weight polypeptide which he provisionally named 'glomin'. Evidence has since been obtained which shows that polypeptide or protein-containing granules are present in mammalian carotid body cells [2,14], but the identity of the polypeptide remains to be established.

The carotid body has a high monoamine content [3,6] and neuroactive peptides are often stored in cells which also contain monoamines [8,9]. Substance P has been found in various peripheral sensory nerve endings [4,5,7] and the present immunofluorescence study was undertaken to determine whether substance P is present in the cat carotid body.

An indirect immunofluorescent procedure was applied as previously described [4]. Carotid bodies of cats anaesthetised with pentobarbitone sodium (42 mg/kg, i.p.) were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 2—4 h at room temperature and then transferred for 1—4 days to the same buffer containing 5% sucrose. Thick sections (10 μm) were obtained in a Dittes cryostat (Dittes, Heidelberg, F.R.G.) and incubated with (1 : 40, v/v) a monoclonal antibody against substance P; immunocharacteristics of which have been described [4] obtained by the hybridoma

strategy [10]. Control preparations were run by incubating alternate sections with some antibody preparation preabsorbed with 200 μg/ml of substance P (Peninsula Labs, U.S.A.). As developing antiserum an antirat IgG, conjugated with FITC (Miles-Yeda) was applied in 1 : 6, v/v. Preparations were observed and photographed in a Leitz Dialux microscope equipped with epifluorescence optics. Micrographs were taken on Kodak T-X film with an Orthomat camera.

In all 14 carotid bodies from 7 cats were examined. Low magnification observations of these organs consistently showed the presence of cells which displayed immunoreactivity following the application of monoclonal antibody (Fig. 1). This immunoreactivity was absent when the material had been incubated with monoclonal antibodies pre-absorbed with substance P (Fig. 2). At higher magnification the non-specific fluorescent spots were seen in the connective tissue space. The positive cells were distributed throughout the carotid body and were sometimes organised in small clusters. No attempt was made to quantify the number of positive cells, but they appeared to comprise approx. 20% of the total population.

Closer observation at higher magnification showed that the positive cells were glomerate in appearance (Fig. 2) and are therefore mainly glomus or type 1 cells. They display a varying degree of immunofluorescence which could be a representation of the physiological state and the amount of substance P-like material intracellularly stored at the moment of fixation. Alternatively, the weaker stained peptide may indicate the presence of an

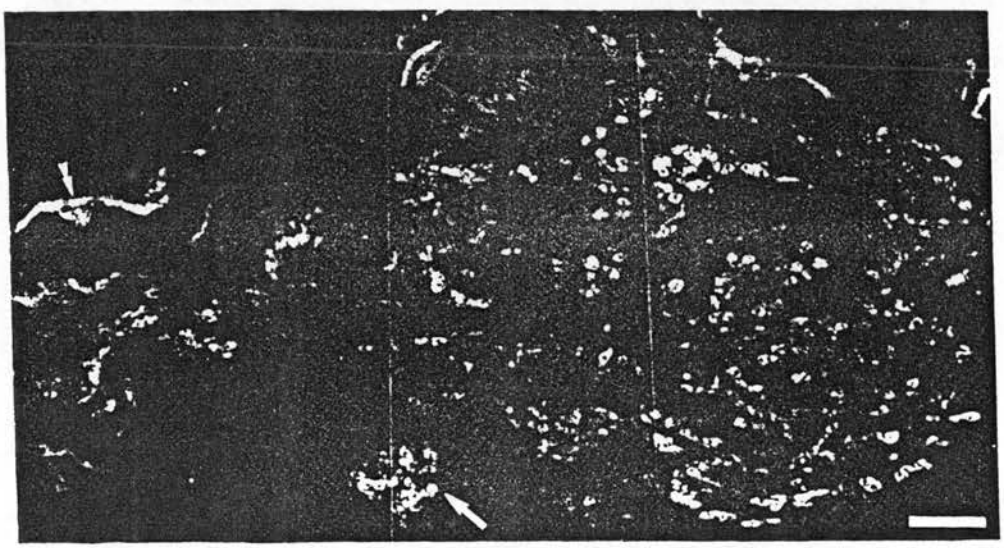

Fig. 1. Low magnification composit micrograph revealing the presence of substance P immunofluorescent glomus cells distributed along the entire carotid body. Double arrow heads indicate unspecific fluorescence in the capsular connective tissue. Substance P immunofluorescent axons are not readily visible at this magnification. Calibration bar = 100 μm.

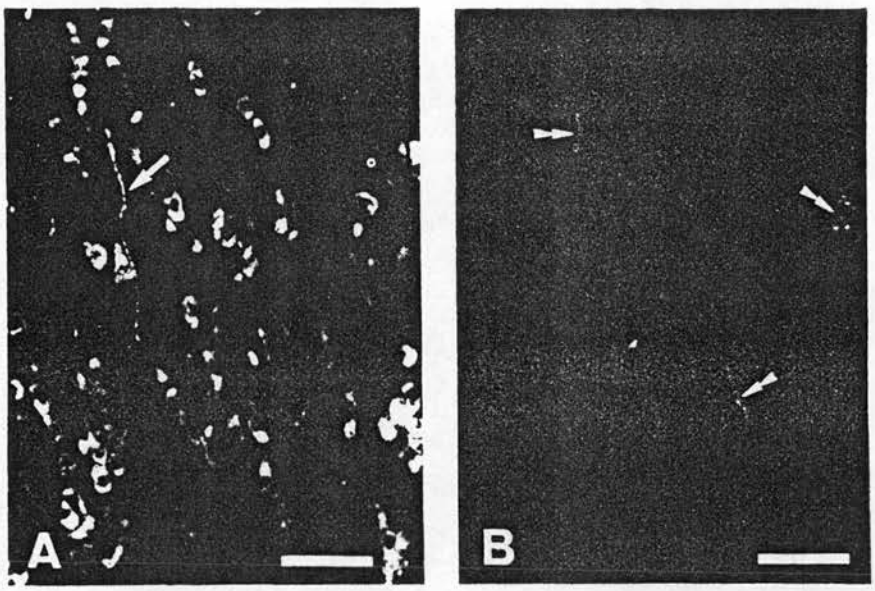

Fig. 2. A: aspect of a zone of the cat carotid body showing several glomerular cells with varying intensity of substance P immunoreactivity as shown in the monoclonal antibody. Arrow shows a long segment of a positive varicose nerve fibre running between substance P positive and negative cells. Calibration bar = 50 μm. B: similar field to Fig. 2A from a sequential section of the cat carotid body incubated with a control solution (monoclonal substance P antibody absorbed with 200 μg/ml of the peptide). Spots showing unspecific fluorescence in the connective tissue are indicated with double arrow heads. Calibration bar = 50 μm.

unknown related peptide which partially cross-reacts with this antibody. At higher magnification the presence of many thin varicose nerve fibres were located in every portion of the carotid body (Fig. 2).

These observations strongly indicate that substance P, or a new polypeptide closely related to it, is stored in the glomus cells of the cat carotid body. Nerve fibers have also been observed to contain this peptide, and it is possible that these are branches of the sensory fibres which form synapses with the glomus cells, the cell bodies being in the petrosal (IX) ganglion. Dopamine, noradrenaline and possibly 5-HT are present in type I cells [3,6] and it will be interesting to determine whether substance P is stored in conjunction with any particular carotid body amine (e.g. as it is in 5-HT-containing CNS neurones [9]).

There is good evidence that carotid body cells (type I and presumably, type II) are essential for the process of chemoreception [15]: the sensory nerve-endings alone are probably not chemosensory, although there is some disagreement about this [1,12]. Whether the cells release a transmitter substance(s) which affects the sensory nerve-ending, or whether they exert a trophic influence on the nerve-endings making them sensitive to physiological

stimuli remains to be established. What role, if any, substance P may have in the process of chemoreception is unknown and needs to be investigated. Exogenous substance P causes a slight, delayed, long-lasting increase in spontaneous chemoreceptor discharge, part of which is probably a primary effect on the chemoreceptors [11].

In conclusion, the present results confirm Pearse's [13] suggestion that a polypeptide is present in the carotid body. 'Glomin' will probably turn out to be a mixture of peptides because preliminary experiments (Cuello and McQueen, unpublished) suggest enkephalins are also present in the cat carotid body. It will be interesting to investigate how substance P in the carotid body interacts with monoamines, whose function also remains to be established, and with other polypeptides since such studies might provide further insight into the process leading to the classical function of the carotid body, namely chemoreception.

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INHIBITORY ACTIONS OF METHIONINE-ENKEPHALIN AND MORPHINE ON THE CAT CAROTID CHEMORECEPTORS

D.S. McQUEEN & J.A. RIBEIRO[1]

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

1 The effects of intracarotid injections of methionine-enkephalin (Met-enkephalin) and morphine on chemoreceptor activity recorded from the peripheral end of a sectioned carotid sinus nerve have been studied in cats anaesthetized with pentobarbitone.

2 Met-enkephalin caused a rapid, powerful, inhibition of spontaneous chemoreceptor discharge, the intensity and duration of which was dose-dependent.

3 Morphine was a less potent inhibitor of spontaneous chemoreceptor discharge, and the inhibition it evoked was rather variable and tended to be biphasic. Low doses of morphine caused a slight increase in discharge.

4 Naloxone (0.2 mg i.c.) slightly increased spontaneous discharge, greatly reduced the chemo-inhibition caused by morphine, and reduced the inhibitory effect of Met-enkephalin. A higher dose of naloxone (0.8 mg) caused a substantial reduction of the Met-enkephalin effect.

5 Chemo-excitation evoked by intracarotid injections of acetylcholine, CO_2-saturated Locke solution, and sodium cyanide were only slightly and somewhat variably reduced following injections of Met-enkephalin, whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to acetylcholine and sodium cyanide were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

6 Responses to acetylcholine and CO_2 were slightly potentiated during infusion of Met-enkephalin (50 μg/min, i.c.) and the response to sodium cyanide was slightly reduced.

7 It is concluded that naloxone-sensitive opiate receptors are present in the cat carotid body; when activated they cause inhibition of spontaneous chemoreceptor discharge. The physiological role of these receptors and the identity of any endogenous ligand remains to be established.

Introduction

Methionine-enkephalin (Met-enkephalin) is a potent inhibitor of spontaneous chemoreceptor discharge in the cat (McQueen, 1979), and the present neuropharmacological study was undertaken to investigate further this action of Met-enkephalin. It was also considered of interest to determine whether morphine has the same effect as Met-enkephalin on the cat carotid chemoreceptors.

Methods

Experiments were performed on ten cats weighing between 2.1 and 3.5 kg, median weight 2.9 kg. They were anaesthetized with pentobarbitone sodium (42 mg/kg i.p. initially, supplemented by i.v. administration of 10% of the initial dose every 1 to 2 h), artificially ventilated and paralysed by gallamine triethiodide (3 mg/kg i.v.). Full details of the experimental techniques have been given previously (McQueen, 1977; Docherty & McQueen, 1978).

Electrical activity of chemoreceptor units (1 to 5 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, passed through a pulse height (window) discriminator, and quantified with the aid of a PDP-8 computer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in modified Locke solution (McQueen, 1977). Drug solutions (0.1 ml) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded, and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; injections were made over 2 s. The catheter was introduced into the common carotid artery via the lingual artery and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. In

[1] Permanent address: Centro de Biologia, Instituto Gulbenkian de Ciência, Oeiras, Portugal.

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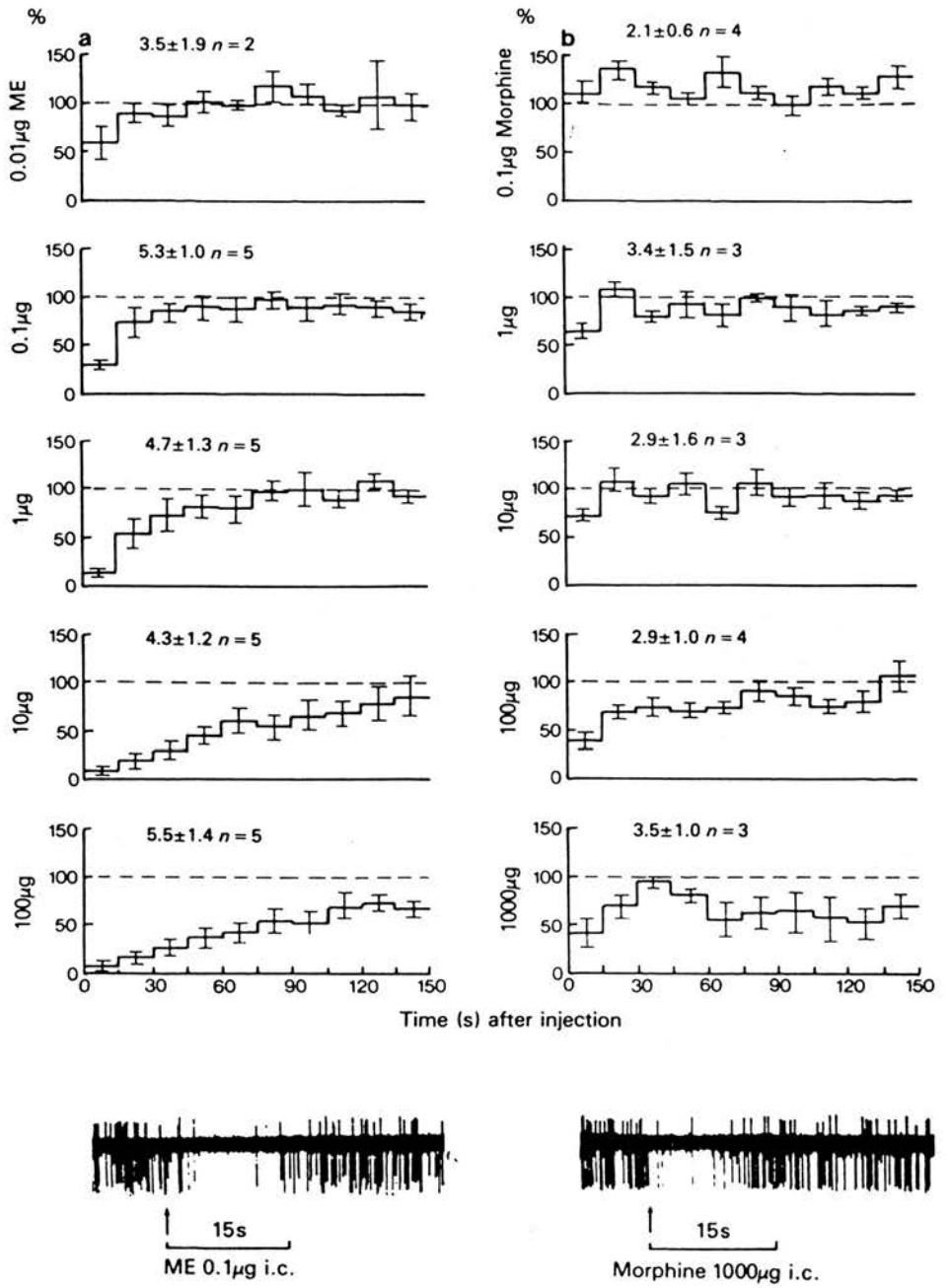

Figure 1 The upper part illustrates the effects of various doses of methionine-enkephalin (ME) (a) and morphine (b) on spontaneous chemoreceptor discharge. Discharge was averaged over 15 s periods following the injection and expressed as a percentage of the averaged discharge in the 15 s pre-injection control period. Data from *n* experiments were pooled and are shown as the mean percentages; vertical lines show s.e. mean. Averaged values (ct/s) ± s.e. mean for the control (100%) periods are given.

The neurograms in the lower part of the figure, taken from one experiment, show the early part of the somewhat similar inhibition of chemoreceptor discharge caused by injecting (arrow) (a) ME 0.1 μg i.c. and (b) morphine 1000 μg i.c.

some experiments a second catheter was positioned in the common carotid artery, this time via the superior thyroid artery, and used for the infusion of drug solutions (0.5 ml/min for 65 s; Braun, Unita).

Drugs used were: morphine sulphate, gallamine triethiodide (May & Baker); sodium cyanide, acetylcholine iodide (B.D.H.); methionine-enkephalin (Uniscience); dopamine hydrochloride (Koch Light) and naloxone hydrochloride (Endo), kindly given to us by Professor W. Feldberg.

Results

Effect of methionine-enkephalin injections on spontaneous chemoreceptor discharge

Met-enkephalin caused a dose-dependent reduction in spontaneous chemoreceptor discharge with a rapid onset, starting within 1 to 2 s of beginning the injection. During the early part of the response there was total inhibition of chemoreceptor discharge, then spontaneous activity returned gradually, reaching control (pre-injection) levels within 30 to 45 s following low doses of Met-enkephalin, but taking up to 5 min to recover after high doses (see Figure 1a). The inhibitory effect was consistent, as can be gauged from the standard errors, and there was no evidence of tachyphylaxis occurring when doses were administered at 7 min intervals. The effect of Met-enkephalin seemed to be potentiated slightly after morphine had been injected. Intravenous injections of Met-enkephalin (10 to 100 μg) also inhibited chemoreceptor discharge, although to a lesser extent and after a longer delay than the same doses by intracarotid injection.

Low doses of Met-enkephalin had little or no effect on blood pressure (BP), whereas higher doses (i.c. or i.v.) caused a fall in BP (see Figure 2).

Effects of morphine injections on spontaneous chemoreceptor discharge

The over-all effect of morphine on spontaneous chemoreceptor discharge was inhibitory, although the lowest dose studied, 0.1 μg, caused a slight increase in discharge (see Figure 1b). Higher doses were associated with an inhibition of discharge which began within 1 to 2 s of starting the injection (Figure 1b) and was dose-related. The initial inhibition lasted for 15 to 45 s after which discharge returned towards pre-injection control levels. There was then a delayed or secondary inhibition of spontaneous discharge, an effect which was most clearly seen after the highest dose of morphine (see Figures 1b, 3a).

Responses to morphine were rather variable, as can be seen from the standard errors, but there was no evidence of tachyphylaxis, and responses to a low dose of morphine injected before and after the highest dose were very similar. The higher doses of morphine caused a fall in BP (see Figure 2).

Effects of naloxone

In an adequately anaesthetized cat, which had received neither Met-enkephalin nor morphine, naloxone (0.2 mg i.c.) caused a slight increase in chemoreceptor discharge and, after a delay of 15 to 45 s, a rise in BP (Figure 2) lasting for at least 30 min. Additional doses of naloxone (0.4, 0.8 and 1.6 mg i.c. at 7 min intervals) had no further effect on chemoreceptor discharge or BP.

When naloxone (0.2 mg i.c.) was injected during experiments in which Met-enkephalin and/or morphine had previously been administered, there was also an increase, albeit somewhat variable, in spontaneous chemoreceptor discharge (Figure 2) and a rise in BP.

Comparison of responses to Met-enkephalin and to morphine obtained before and after injecting naloxone showed that, apart from a slight initial inhibition, the inhibitory action of morphine was virtually abolished, and there was evidence of an over-all increase in discharge. The chemo-inhibitory effect of Met-enkephalin was much reduced (see Figures 2 and 3).

Dose-response data were obtained by expressing the number of impulses in the post-injection period as a percentage of the number of impulses which would have been likely to occur in the same period had the pre-injection control discharge (averaged over 15 to 30 s) continued unaltered, and plotting this value against $\log_{10}$ dose. It can be seen from Figure 3 that naloxone shifts the Met-enkephalin dose-response line to the right, both for 60 s and 150 s post-injection periods. After intracarotid injection of naloxone (0.2 mg), morphine tended to increase discharge over these periods, this effect being inversely related to dose.

A higher dose of naloxone (0.8 mg i.c.) completely abolished the inhibitory action of morphine (1 mg i.c.) and greatly reduced the inhibitory reponse to Met-enkephalin; over-all there was an increase in discharge following lower doses of Met-enkephalin (see Figure 4).

Although naloxone reduced the hypotensive action of Met-enkephalin and morphine, slightly after 0.2 mg i.c. (see Figure 2) and to a greater extent after 0.8 mg i.c., falls in BP were still obtained even when the chemo-inhibitory response had been abolished.

Evoked responses

Injections (i.c.) of acetylcholine (ACh, 50 μg), carbon dioxide-saturated Locke solution (CO_2, 0.3 ml), sodium cyanide (NaCN, 5 μg) and dopamine (5 μg)

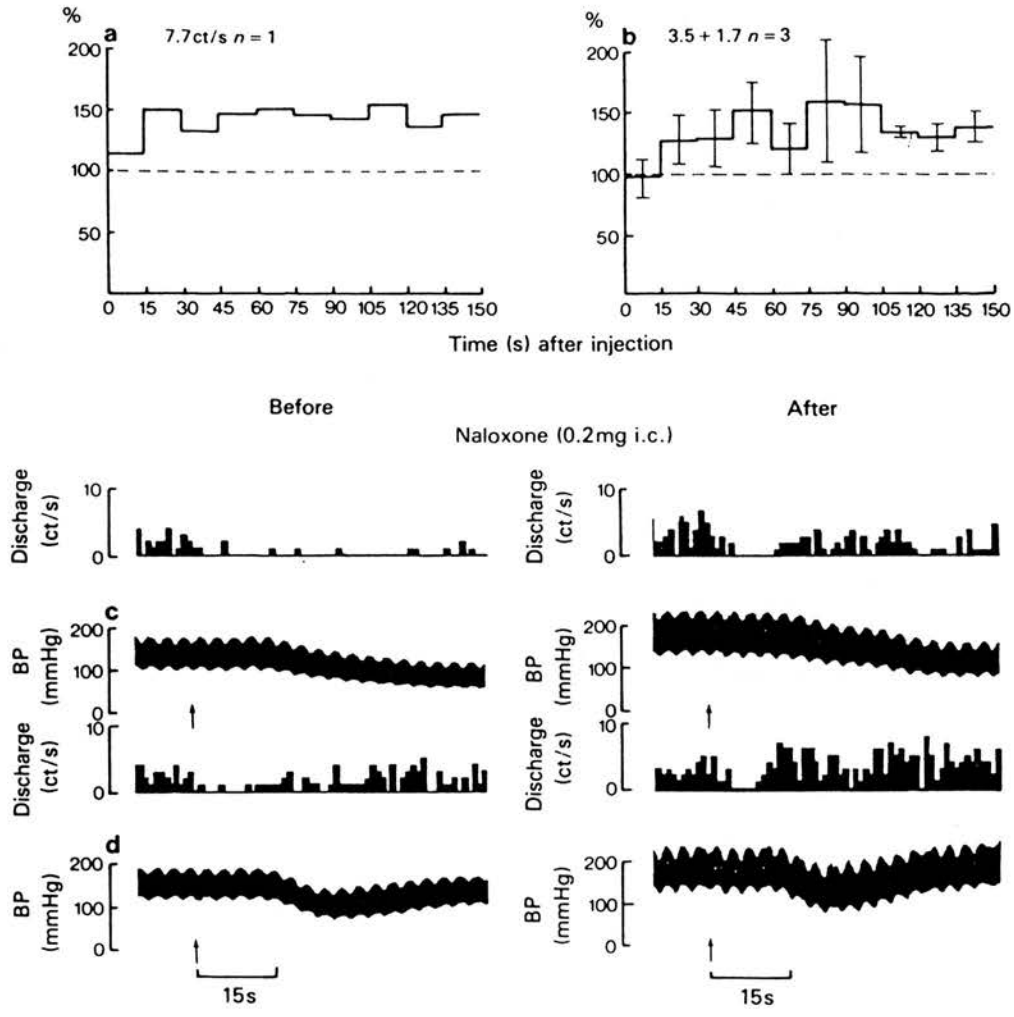

Figure 2 In (a) the effect of naloxone (0.2 mg i.c.) on spontaneous chemoreceptor discharge in an experiment during which no methionine-enkephalin (Met-enkephalin) or morphine had been administered is illustrated; (b) is the averaged spontaneous chemoreceptor discharge from three experiments in which the same dose of naloxone (0.2 mg i.c.) was injected following prior administration of Met-enkephalin and/or morphine. Details as for Figure 1.

Met-enkephalin 100 μg i.c. (c) and morphine 1000 μg i.c. (d) were injected, at the arrows, before and after naloxone (0.2 mg i.c.). Their effects on chemoreceptor discharge and BP are shown, and it can be seen that chemoreceptor inhibition was greatly reduced by naloxone, whereas the hypotension was less affected by this dose of naloxone.

were made before and 5 to 20 min after a series of injections of either Met-enkephalin or morphine. The results obtained (Table 1) showed that the stimulant action of ACh, CO_2, and NaCN were slightly and somewhat variably reduced after Met-enkephalin, whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to ACh and NaCN were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

Responses were also obtained before and during an infusion of Met-enkephalin (50 μg/min i.c.), this dose being sufficient to inhibit spontaneous discharge throughout the infusion period. The effects of ACh and CO_2 were slightly potentiated whereas the response to NaCN was slightly reduced (see Table 1).

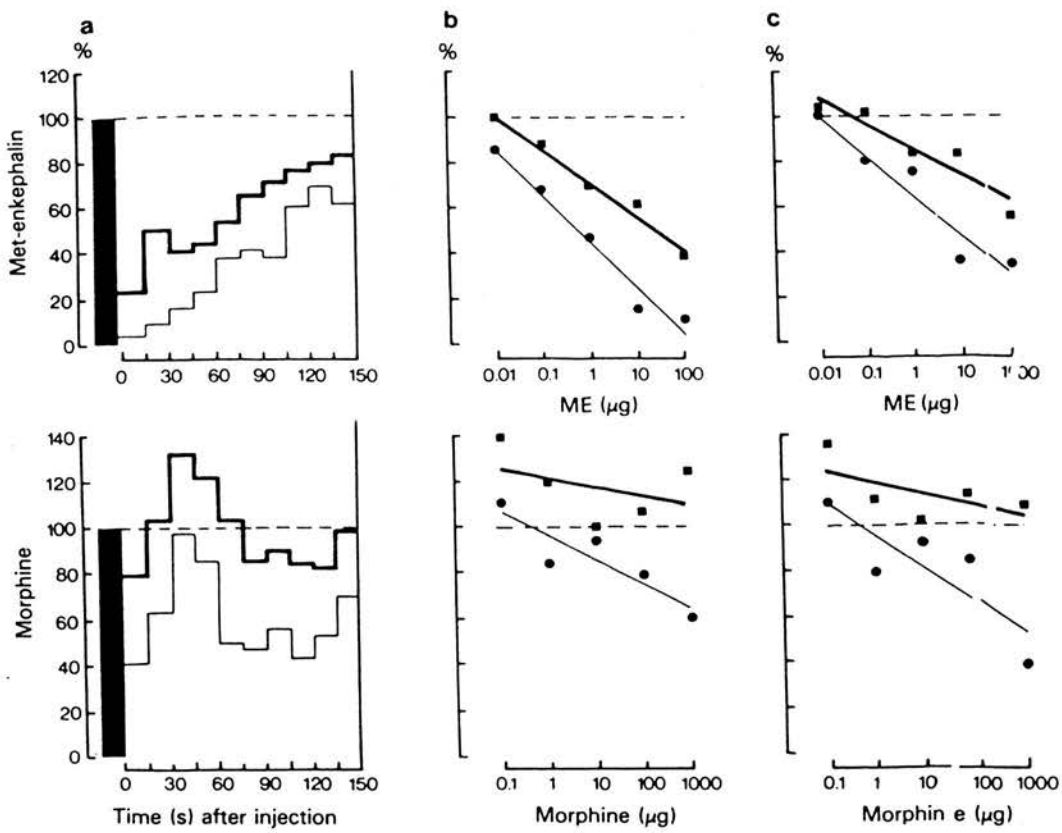

Figure 3 (a) Pooled data showing the effects of methionine-enkephalin (Met-enkephalin) 100 μg (*n* = 3) and morphine 1000 μg (*n* = 2) on spontaneous chemoreceptor discharge before (—) and after (—) injec ting naloxone (0.2 mg i.c.). Black rectangles represent the control discharge (100%), values for Met-enkephalin being 3.7 ± 1.8 ct/s before and 3.1 ± 0.9 after naloxone, the corresponding values for morphine being 3.7 ± 1.7 and 4.9 ± 2.4 ct/s. See Figure 1 for further details. (b) is a plot of the total discharge over the 60 s post-injection period, expressed as a percentage of the total discharge which would have occurred in the same period if control discharge had continued unaltered (100% = dotted line), against $\log_{10}$ dose of methionine-enkephalin (ME) (pooled data fro m three experiments) or morphine (data from a single experiment) before (—) and after (—) naloxone (0.2 mg i.c.). Lines were fitted by the method of least squares. (c) is similar to (b), but the plot is of total discharge in the 150 s post-injection period and this includes the delayed inhibition seen with morphine.

Since spontaneous discharge was suppressed it was not possible, under these conditions, to investigate the effect of Met-enkephalin on the inhibitory response evoked by dopamine.

Responses to ACh, NaCN, CO_2 and dopamine were not appreciably affected by naloxone (0.2 mg i.c.).

Discussion

Our results indicate that enkephalin inhibited spontaneous chemoreceptor discharge by acting on naloxone-sensitive receptors in the carotid body. Morphine also acted at these receptors, but it was less potent than Met-enkephalin and c aused a more variable inhibition which tended to be biphasic.

Although Met-enkephalin and morphine were able to inhibit spontaneous chemorece ptor discharge, they only slightly reduced excitatory r esponses evoked by ACh and NaCN, and potentiate d dopamine-induced inhibition. These were long-term effects since the responses were not studied until 5 to 20 min after injections of Met-enkephalin or morphine. When responses were studied during an infusion of Met-enkephalin sufficient to reduce spontaneous chemoreceptor discharge substantially, the excitatory effect of NaCN was slightly reduced whereas responses to ACh and CO_2 were potentiated. These single-dose studies are difficult to interpret, particularly since

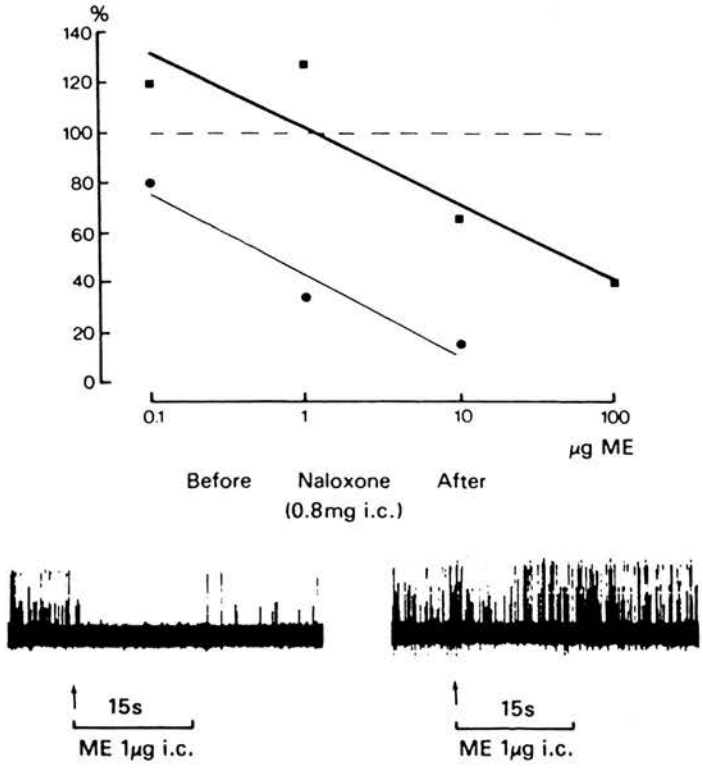

Figure 4 Effects of methionine-enkephalin (ME) on chemoreceptor discharge (plotted as a percentage of the total discharge in the 150 s post-injection period/control discharge × 150 s) before (—) and after (—) injecting naloxone (0.8 mg i.c.). Lines were fitted by the method of least squares. See Figure 3 for further details. Following this dose of naloxone, low doses of Met-enkephalin no longer inhibited chemoreceptor discharge; the tendency was for discharge to be increased. This can be clearly seen in the neurograms which show the effect of Met-enkephalin (1 μg i.c.), injected at the arrow, before and after naloxone (0.8 mg i.c.).

Table 1 Effects of methionine-enkephalin (Met-enkephalin) and morphine on evoked responses

Intracarotid injections of:	*ACh* (50 μg)	*CO_2-Locke* (0.3 ml)	*NaCN* (5 μg)	*Dopamine* (5 μg)	n
A Following morphine injections	83 ± 19	134 ± 11	89 ± 1	159 ± 61	3
B Following Met-enkephalin injections	77 ± 16	72 ± 17	63 ± 10	167 ± 24	5
C During Met-enkephalin infusions (50 μg/min i.c.)	137 ± 23	122 ± 9	84 ± 23	—	2

Pooled data from all experiments showing chemoreceptor responses ($\Delta\Sigma x$) (A) following injections of morphine and (B) following injections of Met-enkephalin. The results are expressed as mean percentages ± s.e. mean of the responses to the same doses administered before morphine or Met-enkephalin (i.e. pre-injection response = 100%). Injections were also made 60 s after starting a 65 s infusion of Met-enkephalin and responses evoked compared with pre-infusion values (C).

$\Delta\Sigma x = \Sigma x$ (total spike count during response period, t s) $- (\bar{x}.t)$ where $\bar{x}$ is the average pre-injection (control) discharge in ct/s.

Met-enkephalin may alter blood flow through the carotid body, but the important point is that whereas Met-enkephalin can reduce spontaneous chemoreceptor discharge, it has little effect on responses to intense stimuli of short duration. In the present experiments, the local concentration of Met-enkephalin/morphine may have been high enough to suppress the resting discharge but not that evoked by ACh, NaCN or CO_2.

Injection of the specific opiate antagonist, naloxone (see Sawynok, Pinsky & Labella 1979 for a review), slightly increased chemoreceptor discharge, an effect that was not simply due to reversal of residual Met-enkephalin or morphine chemo-depression because it was also observed when neither substance had been administered. This may mean that there is some tonic inhibition of chemoreceptor discharge by an opioid; alternatively, the effect could be secondary to changes (e.g. in BP) induced by naloxone acting elsewhere. The dose of naloxone used (0.2 mg) is adequate for reversing the fall in BP caused by morphine in cats (Feldberg & Wei, 1977; McQueen, unpublished observations) and for reversing the action of enkephalins in the cat substantia gelatinosa (Duggan, Hall & Headley, 1977). In the present experiments it reduced the chemoreceptor inhibition caused by Met-enkephalin and, to a greater extent, that caused by morphine. It is known that inhibition of neuronal firing caused by morphine is more readily antagonized by naloxone than is that caused by opioid peptides (North, 1979). Increasing the dose of naloxone to 0.8 mg caused an even greater reduction of the Met-enkephalin-induced inhibition of chemoreceptor discharge, and virtually abolished the morphine effect. Higher doses of naloxone could have been studied, but we were concerned that they might have exerted non-specific actions, or reversed the anaesthetic (Fürst, Foldes & Knoll, 1977; Arndt & Freye, 1979; Sawynok *et al.*, 1979). It seems reasonable to conclude that most of the chemoreceptor inhibition results from actions of Met-enkephalin and morphine on naloxone-sensitive receptors in the carotid body.

Low doses of morphine tended to increase spontaneous chemoreceptor discharge, an effect that was potentiated and also obtained with higher doses of morphine, as well as low doses of Met-enkephalin, after naloxone. Landgren, Liljestrand & Zotterman (1952) found that an intracarotid injection of 3 mg morphine hydrochloride in cats caused a moderate increase in small action potentials (probably chemoreceptors) recorded from the sinus nerve, and Eyzaguirre & Zapata (1968) showed that morphine caused a transient increase in discharge recorded from the *in vitro* carotid body preparation. Whether the excitation seen in the present study resulted from an action on naloxone-insensitive opiate receptors, or was caused by morphine/Met-enkephalin influencing substances in the carotid body, as occurs in other tissues (e.g. ACh (Paton, 1957), noradrenaline (Szerb, 1961; Snyder & Childers, 1979), 5-HT, or dopamine (Loh, Brase, Sampath-Khanna, Mar, Way & Li, 1976)), requires further investigation. So does the biphasic nature of the inhibitory response to morphine; could vascular changes be responsible for the transient return of discharge to control levels?

High doses of Met-enkephalin and morphine caused a fall in BP, and it is possible that some of the changes in chemoreceptor discharge might result from changes in blood flow through the carotid body. However, the fact that chemo-inhibitory actions of Met-enkephalin and morphine, (a) started within 1 to 2 s of the injection, (b) occurred following low doses which had no effect on BP and, (c) were greatly reduced by naloxone in doses which only slightly reduced the hypotensive effect, all argue against vascular effects contributing much to the inhibition, at least as far as the early part of the response is concerned. Further information could be obtained by studying the effect of Met-enkephalin on the *in vitro* carotid body preparation (Eyzaguirre & Lewin, 1961), which would eliminate the vascular complications.

The chemo-inhibitory effect of low doses of Met-enkephalin was very similar to that obtained with dopamine, (5 μg i.c.), although it should be noted that Met-enkephalin is 10 to 100 times more potent on a molar basis. However, despite the similarities, it is unlikely that Met-enkephalin acts directly, or, by releasing dopamine within the carotid body, indirectly at a dopamine receptor, because α-flupenthixol blocks the inhibitory action of exogenous dopamine (Docherty & McQueen, 1978) without affecting the inhibitory response to Met-enkephalin (McQueen, 1979).

Substance P is present in the cat carotid body (Cuello & McQueen, 1980) and causes an increase in chemoreceptor discharge on intracarotid injection (McQueen, 1980). It may be that Met-enkephalin is acting to inhibit the release of substance P within the carotid body in the same way as has been shown to occur in the CNS (Jessel & Iversen, 1977), the mechanism probably involving an action of Met-enkephalin on Ca^{2+} channels (Mudge, Leeman & Fischbach, 1979).

It has been suggested that adenosine might be the mediator of the neuro-inhibitory action of opiates (Sawynok & Jhamandas 1976; Stone & Perkins, 1979). Both adenosine (see Ribeiro, 1978) and morphine (Henderson, Hughes & Kosterlitz, 1975) decrease transmitter release in the central and peripheral nervous systems, and adenosine (Ribeiro, Sá-Almeida & Namorado, 1979), morphine (Guerrero-Munoz, Cerreta, Guerrero & Way, 1979), and β-endorphin (Guerrero-Munoz, Guerrero, Way & Li, 1979) decrease the uptake of calcium by synapto-

somes; Ca^{2+} is known to be involved in transmitter release (e.g. Katz & Miledi, 1968). Opioids might depress chemoreceptor activity by inhibiting the entry of Ca^{2+} needed for the release of putative sensory excitatory transmitter(s). Whether adenosine is the mediator of the Met-enkephalin or morphine-induced inhibition, and whether Met-enkephalin interacts with other substances in the carotid body (e.g. substance P, noradrenaline, 5-hydroxytryptamine, dopamine, ACh) requires investigation. Preliminary results (Cuello & McQueen, unpublished observations) suggest that enkephalin-like material is present in the carotid body.

In conclusion, the present results provide pharmacological evidence for the presence of an opiate receptor, or receptors, in the cat carotid body. What type of receptor this is (e.g. Lord, Waterfield, Hughes & Kosterlitz, 1977), where in the carotid body it is located, what the endogenous ligand is and where it originates, and the circumstances under which it is released, all need to be investigated before one can determine whether Met-enkephalin or other opioid peptides have a role as neurotransmitters or neuromodulators (Kosterlitz & Hughes, 1975; Snyder & Childers, 1979) in the cat carotid body chemoreceptors.

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EFFECTS OF SOME POLYPEPTIDES ON CAROTID CHEMORECEPTOR ACTIVITY

D.S. McQueen, Department of Pharmacology, University of Edinburgh.

Key Words - Glomin, substance P, neurotensin, enkephalin, bradykinin, somatostatin.

Pearse (22) suggested that carotid body type I cells secrete a low molecular weight polypeptide (glomin). Histological evidence has since been obtained which indicates that polypeptide or protein-containing granules are present in mammalian carotid body cells (3, 23).

The evidence that a polypeptide is present in the carotid body, together with the fact that substance P (SP) - like material is associated with some peripheral sensory nerve endings (7, 12) prompted the present investigation into the effects of SP, and some other polypeptides associated with the nervous system, on the cat carotid chemoreceptors.

METHODS

Experiments were performed on cats anaesthetised with pentobarbitone sodium (42 mg/kg i.p.), artificially ventilated with air and, usually, paralysed by gallamine (3 mg/kg i.v.). Full details of the experimental technique have been described previously (19) and only a brief description is given here. A sinus nerve was cut centrally and electrical activity recorded from 1 - 5 chemoreceptor units recorded from filaments of the peripheral nerve using bipolar electrodes. The ganglioglomerular nerves were cut. Nerve activity was recorded on tape and subsequently analysed with the aid of a pulse height (window) discriminator and a PDP-8 computer. Responses were expressed as a percentage of the control, and data from different experiments pooled.

Drugs were injected (0.1 ml + 0.2 ml wash over 2 sec) or infused (0.5 ml/min for 65 sec) into the ipsilateral common

carotid artery via catheters in either the lingual or superior thyroid arteries. The following polypeptides were used, dissolved in modified Locke solution (19): substance P, SP, neurotensin, NT, (Sigma); Metenkephalin, ENK, (Uniscience); somatostatin, SS, (U.C.B.); eledoisin-related peptide (Calbiochem); bradykinin, BK, (Protein Research Foundation); angiotensin amide, ANGIO, (Hypertension - Ciba); A.D.H., (Pitressin, Parke-Davis).

RESULTS

Spontaneous chemoreceptor discharge. Dose-response studies established that the effects of the injected polypeptides were dose-related; results obtained with the highest doses studied are summarised in Fig. 1.

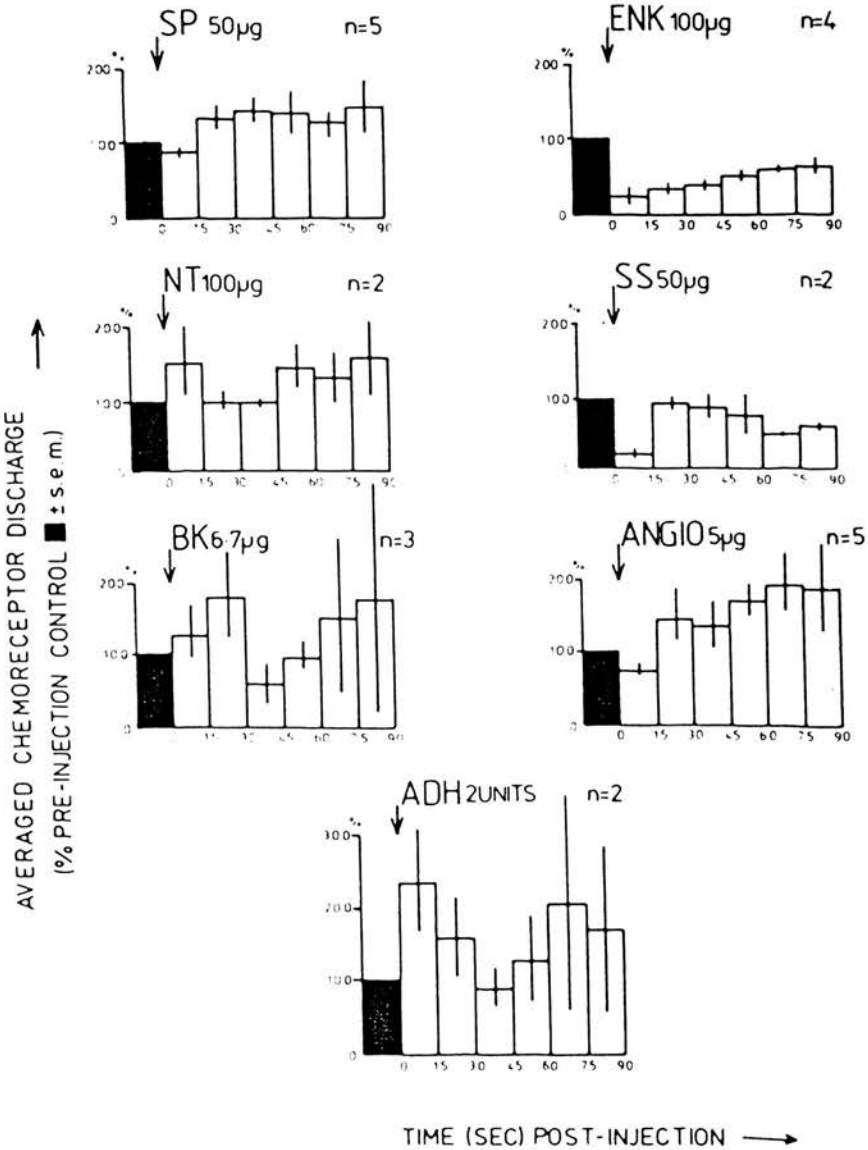

Fig. 1. Pooled data showing the effects of high doses of polypeptides on spontaneous chemoreceptor discharge. Discharge was averaged over 15 sec periods and expressed as a percentage of the pre-injection discharge. n = number of recordings.

After a delay of 1 - 15 sec following injection, SP caused a long-lasting (45 - 300 sec) dose-dependent increase in discharge. Eledoisin-related peptide had very similar actions. In contrast, ENK caused an immediate dose-dependent reduction in discharge lasting for 15 - 150 sec (see Fig. 2). Both SP and ENK caused a fall in B.P.

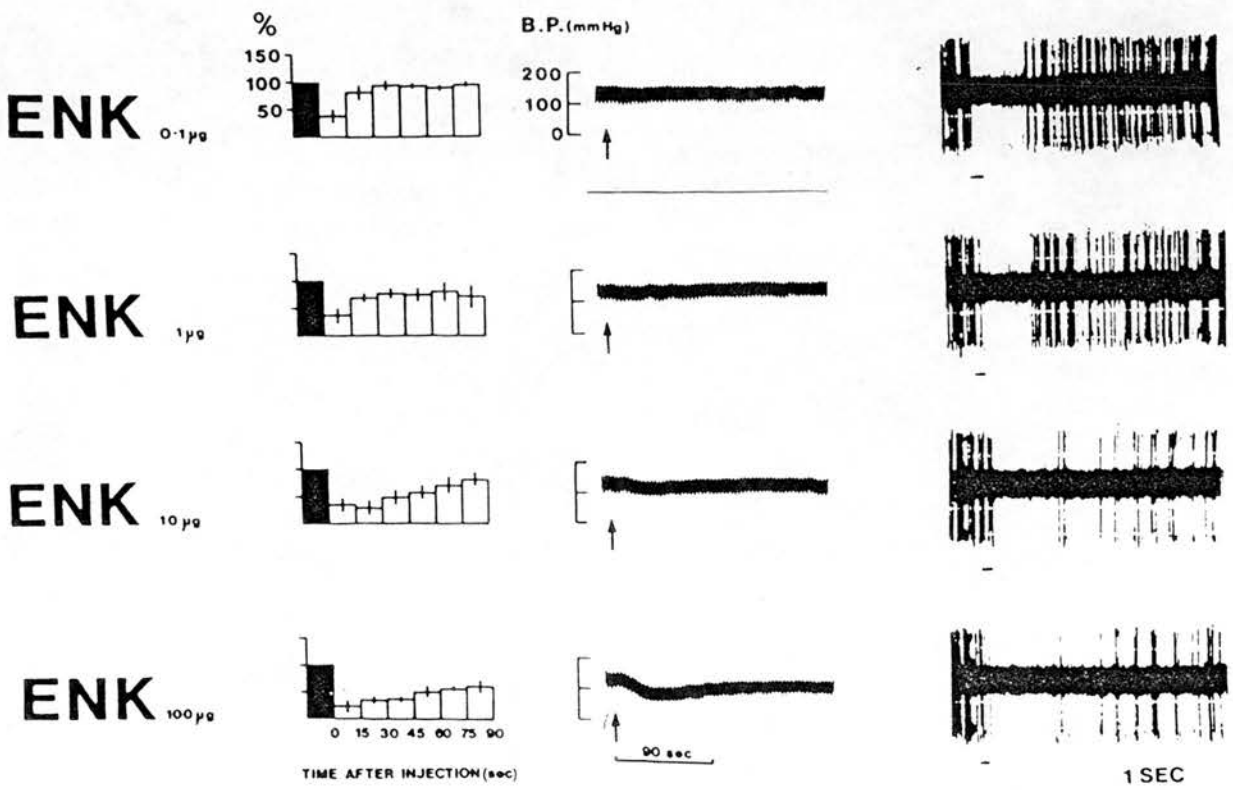

Fig. 2. Pooled data from Met-enkephalin experiments (n = 4) illustrating the dose-dependent nature of the inhibition.

B.P. and discharge of a single chemoreceptor unit from one of the experiments are also shown. Details as for Fig. 1. Horizontal bar is the injection marker.

SS was associated with a short-lasting inhibition of spontaneous chemoreceptor discharge followed, about 30 sec later, by a longer-lasting reduction; B.P. fell slightly. NT caused a slight and variable increase in chemoreceptor discharge lasting for about 15 sec; there was a very slight increase in B.P., followed by a fall. BK, ANGIO and A.D.H. had rather variable effects on chemoreceptor discharge and were associated with marked changes in B.P.

Responses to ACh and NaCN. A comparison was made of responses to these chemoreceptor stimulants obtained before a 65 sec infusion of polypeptide and 60 sec after starting the infusion. Responses evoked by ACh (50 µg) were slightly potentiated during ENK infusion and reduced by BK, SP, SS and NT. Responses to NaCN (5 µg) were slightly potentiated by SS and SP, but reduced during infusions of ENK, BK and NT.

- Flupenthixol and Naloxone. Dose-response data were obtained for ENK injected before and after administering sufficient α-flupenthixol to block the inhibitory action of DA (5 g) on chemoreceptor discharge. The results are shown in Fig. 3. Responses to ENK and DA were also obtained before and after injecting the opiate antagonist naloxone (0.2 mg i.v.). The inhibitory effect of ENK was greatly reduced by naloxone, whereas that of DA was unaffected.

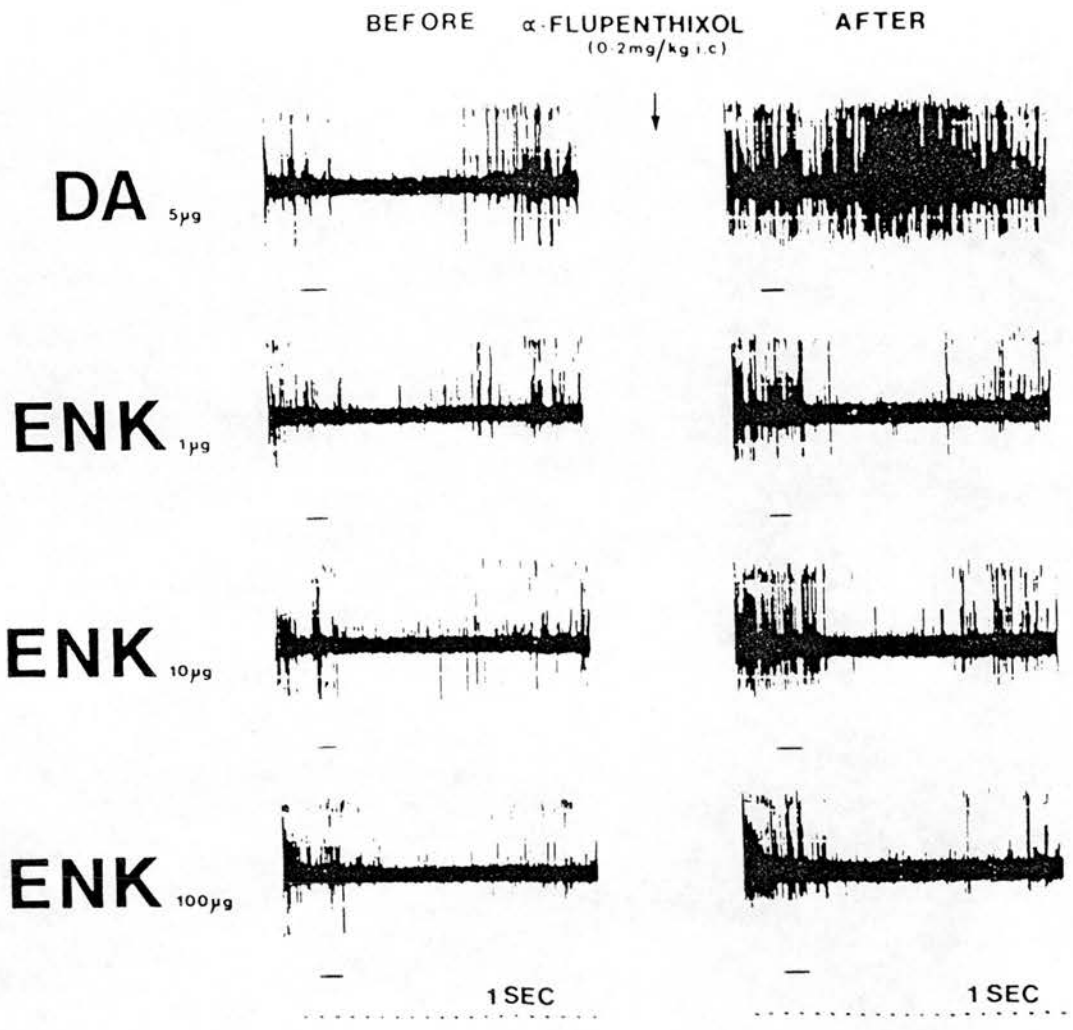

Fig. 3. Recording of multi-unit chemoreceptor discharge showing the effects of the DA antagonist α-flupenthixol on inhibitory responses to DA and ENK. Horizontal bar is the injection marker.

DISCUSSION

High doses of all the polypeptides investigated had some effect on spontaneous chemoreceptor discharge, which is perhaps not surprising in view of the vascular effects which generally accompanied polypeptide administration. None of them caused the intense chemoreceptor stimulation associated with ACh, NaCN, CO_2 or hypoxia. SP did evoke a slight but consistent increase in discharge after a delay of 5 - 15 sec, an effect which did not seem to be correlated with the fall in B.P. It is difficult to establish whether this is a

direct effect of SP, partly because of the lack of a specific SP antagonist (4, 16). The finding that synthetic SP excites certain peripheral sensory nerves (15) has not been confirmed (17). NT caused a more transient and variable increase in chemoreceptor discharge.

Infusions of SP reduced the chemoreceptor stimulant effect of ACh but potentiated that of NaCN. SP is known to have a nicotinic-blocking action (18, 24), and slight nicotinic block reduces the chemoreceptor response to ACh and potentiates that to NaCN (19). The present results are compatible with SP having a slight nicotinic-blocking action on the carotid chemoreceptors (20).

The effect of ENK on the chemoreceptors was quite dramatic. There was an immediate reduction of spontaneous discharge, the intensity and duration of which was dose-dependent. The effect of low doses of ENK was very similar to that of DA (5 g), with the polypeptide being much more potent on a molar basis. Although the inhibitory effect was similar to that caused by DA, it was not affected by a dose of the DA antagonist α-flupenthixol (8) sufficient to block the response to DA. However, the ENK inhibition of chemoreceptor activity was much reduced by the opiate antagonist naloxone, whereas DA inhibition was unaffected. This evidence suggests that an opiate receptor is present in the cat carotid body.

The distribution of opiate binding sites in the brain stem and spinal cord shows striking similarity to sites at which both ENK and SP are located (1, 6, 14). SP generally excites neuronal activity in responsive cells, after a delay (11, 16), whereas ENK usually inhibits neuronal discharge (10), which is the same pattern as observed with the chemoreceptors. ENK can also reduce the release of SP from primary afferent fibres (14), possibly by affecting Ca^{++} (21). SP has been shown to be present in some central 5-HT-containing neurones (13) and 5-HT is located in the cat carotid body (5).

It is tempting to speculate about possible interactions between ENK, SP, and catecholamines in the carotid body, and also about whether SP might be stored with 5-HT in this organ. Is SP Pearse's 'glomin': is there more than one polypeptide in the carotid body? It would seem best to await the outcome of histological studies aimed at establishing whether these polypeptides are present in the cat carotid body before making too much of the present results with exogenous ENK and SP. In any event, the physiological role of polypeptides in the nervous system is very much a matter for debate (2, 9, 10) and further investigation.

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DISCUSSION

NISHI: I have also injected substance P and enkephalin. In the case of substance P, 100 mgm caused only a slight increase in chemoreceptor discharge. In the case of enkephalin, I could get a very dramatic effect, as you observed, and also bradykinin is not so specific, most of the nerve ending is sensitive to bradykinin.

MCQUEEN: That is why I have said I concentrated mainly on enkephalin and substance P; I quite agree with you about the specificity of bradykinin, and that is why I have not spent much time on it.

MAJCHERCZYK: Did you cut the sympathetic supply to the carotid body.

MCQUEEN: Yes.

MAJCHERCZYK: Now, do you think it would be better if you had done the experiment the way Michael Purves did some time ago, that is, to perfuse the carotid body at constant pressure. Because, obviously, as you have mentioned, you get drops in blood pressure and that might have had some interaction with your results, and the third thing is that we found that bradykinin has no effect, or very little effect on chemoreceptor activity. However, there was an interesting feature. When we injected bradykinin into the carotid artery above the carotid body, mainly to the cerebral circulation we could observe a very marked increase in efferent activity going to the sinus nerves. If you recorded the chemoreceptor activity in the otherwise intact nerve with preserved efferent innervation, the response to the injection of bradykinin could have been different.

MCQUEEN: Perhaps. The question about flow; I've never been very happy about perfusing the carotid body at constant flow because I don't know how you do it. I mean, you can pump the region with a constant flow but that doesn't mean the carotid body will be perfused in constant flow.

ZAPATA: If I remember correctly there are some old clinical observations that morphine treated patients when they receive, by accident I should say, oxygen, become apnoeic. So that is proof that their respiration was maintained by chemoreceptors. If there are these opiate-receptors in the carotid body it would mean that morphine is decreasing respiration both at the central and peripheral levels. Is that true?

MCQUEEN: I would like to refrain from speculating on that

because I haven't done morphine experiments yet. You have to be a bit cautious because the actions of the enkephalins and for that matter the endorphins may not be the same as those opiates; there may be different receptors.

LAHIRI: We happen to have this information, Dr. Zapata, on the effects of morphine on ventilation. The effect of morphine on the carotid chemoreceptor activity is small compared to the effect of morphine on ventilation and therefore the central effect seems to be much greater.

MCQUEEN: I think one has to remember also that for example, Gaddum used to use it to block some of the effects of hydroxytryptamine so one has to be a bit cautious about whether or not the enkephalins do this sort of thing.

SMITH: Have you examined the effects of naloxone without first administering enkephalin? In other words, you get stimulation after enkephalin but if you simply give naloxone alone do you get stimulation also?

MCQUEEN: The answer is that experiment was done only last week and I haven't had a chance to analyse it.

FITZGERALD: There is in the hypothalamus precedent for V.I.P. modulating dopaminergic neurones. Is there any precedent in the central nervous system for dopamine enkephalin interaction?

MCQUEEN: There is certainly some evidence but I think it is probably too early to speculate about this.

Comparison of the depressant effects of leucine- and methionine-enkephalin on spontaneous chemoreceptor activity in cats

D.S. McQUEEN & J.A. RIBEIRO[1]

Department of Pharmacology, University of Edinburgh, Edinburgh, EH8 9JZ.

There is evidence that methionine-enkephalin (met-ENK) and leucine-enkephalin (leu-ENK) are present in cat carotid body type I cells (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980). Intra-carotid injection of met-ENK decreases spontaneous chemoreceptor discharge in cats (McQueen, 1979; McQueen & Ribeiro, 1980). The present study was undertaken to investigate the effects of leu-ENK on the carotid chemoreceptors and to compare them with those of met-ENK.

Experiments were performed on cats anaesthetized with pentobarbitone (42 mg/kg i.p., supplemented every 1–2 h). The animals were artificially ventilated with air and paralysed by gallamine (3 mg/kg i.v.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve (McQueen, 1977). In the majority of experiments the ganglioglomerular (sympathetic) nerves were cut. Drug solutions were injected into the ipsilateral common carotid artery (i.c.) over a 2 s period.

Leu-ENK and met-ENK both decreased spontaneous chemoreceptor discharge frequency in a dose-dependent manner, being of similar potency (see Figure 1). The intensity and duration of the depression evoked by both peptides were reduced after naloxone (0.2–0.4 mg i.c.). Similar responses were obtained in all the experiments, regardless of whether or not the sympathetic nerve supply to the carotid body had been cut.

These results suggest that leu-ENK and met-ENK depress spontaneous chemoreceptor discharge by acting on opiate receptors in the carotid body. Since the enkephalins have potent effects on chemoreceptor discharge and are present in the carotid body, they may function there as neurotransmitters or neuromodulators. Further studies are needed to establish their physiological roles in the carotid body and to identify the opiate receptor(s) (Hughes, Kosterlitz, McKnight, Sosa, Lord & Waterfield, 1978).

[1] On leave from Instituto Gulbenkian de Ciência, Oeiras, Portugal.

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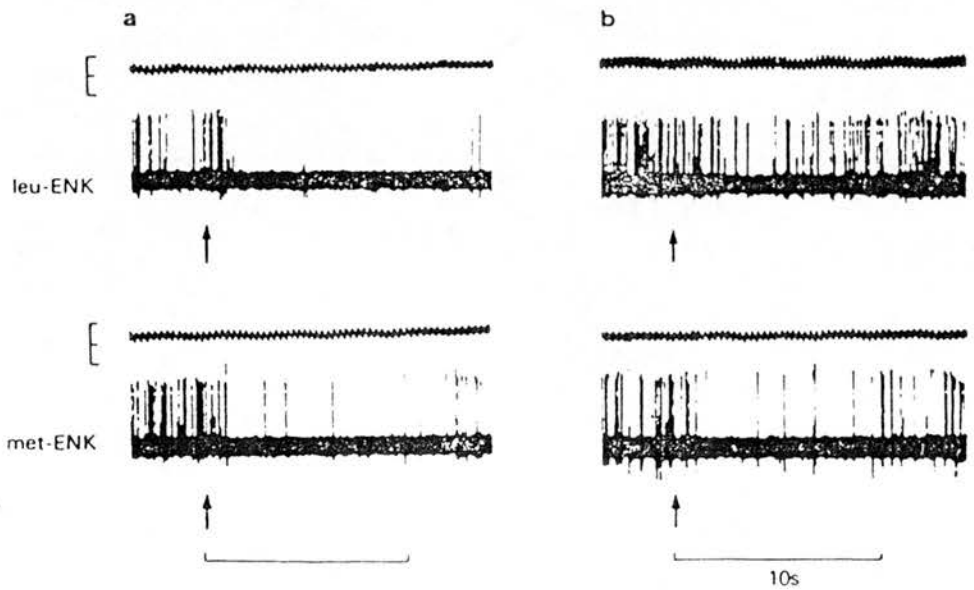

Figure 1 Effects of i.c. injections (arrows) of leu-ENK (10 μg) and met-ENK (10 μg) on spontaneous chemoreceptor activity before (*a*) and after (*b*) naloxone (0.4 mg i.c.). leu-ENK was injected 5 min after naloxone and met-ENK 10 min later. The upper part of each panel shows the femoral arterial B.P. (calibration 0, 100, 200 mmHg), and the lower part the neurogram.

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EFFECTS OF β-ENDORPHIN, VASOACTIVE INTESTINAL POLYPEPTIDE AND CHOLECYSTOKININ OCTAPEPTIDE ON CAT CAROTID CHEMORECEPTOR ACTIVITY

D. S. McQUEEN AND J. A. RIBEIRO*

Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh EH8 9JZ

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SUMMARY

The effects of β-endorphin, vasoactive intestinal polypeptide (VIP) and cholecystokinin octapeptide (CCK-8) on carotid chemoreceptor activity have been investigated in cats anaesthetized with pentobarbitone. Spontaneous chemoreceptor discharge was decreased by intracarotid injection of β-endorphin and by low doses of VIP, whereas it was increased by CCK-8 and higher doses of VIP, these effects being relatively long-lasting and often associated with changes in systemic blood pressure. The chemoexcitation evoked by acetylcholine and sodium cyanide was reduced during intracarotid infusion of any of the three peptides studied, and that caused by CO_2-saturated Locke solution was reduced by β-endorphin, largely unaltered by VIP and variably affected by CCK-8. The inhibitory effect of β-endorphin was greatly reduced by naloxone, implying that it probably involved actions at naloxone-sensitive opiate receptors in the carotid body. Substance P was unable to overcome the chemoinhibitory effect of methionine enkephalin. Possible functions of polypeptides in the carotid body are discussed.

INTRODUCTION

The ability of some polypeptides to modify spontaneous chemoreceptor discharge when injected close-arterial to the cat carotid body has been described (McQueen, 1979, 1980). Since then evidence has been presented which shows that substance P (SP), the enkephalins and vasoactive intestinal polypeptide (VIP), or closely related immunoreactive substances, are present in the cat carotid body (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Cuello & McQueen, 1980; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980).

The present neuropharmacological study was undertaken to investigate further the actions of various polypeptides, including those identified as being present in the carotid body, on the cat carotid chemoreceptors with a view to obtaining some insight into the physiological role of these substances in the carotid body.

METHODS

Experiments were performed on nine cats weighing between 3·0 and 4·4 kg, median weight 3·5 kg. They were anaesthetized with pentobarbitone sodium (42 mg.kg^{-1} I.P.), supplemented as required during the experiments, artificially ventilated with air and paralysed with gallamine (3 mg.kg^{-1} I.V.). Full details for most of the experiment procedures have been given previously (McQueen, 1977; Docherty & McQueen, 1978) and only a brief description follows.

The lingual and superior thyroid arteries ipsilateral to the sinus nerve from which recordings were

* On leave from Instituto Gulbenkian de Ciência, Oeiras, Portugal.

obtained were both cannulated, the catheter tips being positioned in the common carotid artery; blood pressure was recorded from a femoral artery. Electrical activity of chemoreceptor units (1–5 units) was recorded from filaments of the peripheral end of a sectional sinus nerve, stored on FM tape, passed through a pulse height (window) discriminator and quantified with the aid of a PDP-8 computer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in either modified Locke solution (see McQueen, 1977) or 0·9% w/v aqueous sodium chloride solution, except β-endorphin which was initially dissolved in 0·5% aqueous bovine serum albumin solution, then diluted with saline. *Injections* were made in a volume of 0·1 ml into the lingual catheter and washed in with 0·2 ml Locke solution which had been bubbled with 5% CO_2:95% air in a water bath at 37 °C; they were made over a 2 s period. *Infusions* were made into the common carotid artery via the thyroid catheter at a rate of 0·1 ml.min^{-1}, using a Unita pump (Braun), and lasted for 5–10 min; the catheter dead-space was 0·2 ml.

Drugs used were: pentobarbitone sodium, gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide (B.D.H.), dopamine hydrochloride (Koch Light), substance P, methionine enkephalin formate (Sigma), cholecystokinin octapeptide non-sulphated (Peninsula Labs), naloxone hydrochloride (Endo), human β-endorphin (Aalton Bio Reagents, Dublin), porcine β-endorphin (kindly given to us by Dr D. Smyth, National Institute for Medical Research, London) and vasoactive intestinal polypeptide (kindly given to us by Dr S. I. Said, University of Texas Health Science Center, Dallas, U.S.A.).

RESULTS

β-endorphin

In three experiments the effects of β-endorphin on chemoreceptor activity were examined. Since there was no appreciable difference between the actions of human and porcine β-endorphin, results have been expressed simply as responses to β-endorphin.

Injections of β-endorphin. Injections of β-endorphin (0·1–50 μg I.C.) caused a decrease in spontaneous chemoreceptor discharge (see Fig. 1 *B* and *C*). The response lasted for about 3 min following the highest dose investigated (50 μg) and was associated with a very slight fall in systemic blood pressure (Fig. 1 *A*). Discharge was averaged over the 60 s period immediately following β-endorphin injection and expressed as a percentage of the averaged pre-injection (control) discharge (Fig. 1 *D*). The results showed that the decrease in discharge was dose-dependent and, from consideration of discharge during the second minute following the injection (Fig. 1 *E*), fairly long-lasting. The reason for the somewhat variable nature of the response, reflected in the scatter of points about the lines, was that responses to β-endorphin were often bi- or triphasic; there was an initial decrease followed by a return to control level, or to discharge frequencies greater than control, then a delayed decrease in spontaneous discharge (see Fig. 2 *A*). The overall effect was similar to that evoked by morphine (McQueen & Ribeiro, 1980).

Following administration of the opiate antagonist naloxone (0·4 mg I.C.) β-endorphin caused only a slight decrease in spontaneous chemoreceptor discharge, the main effect being a rather variable increase in discharge (Fig. 2 *B*) which was not abolished by additional doses of naloxone.

Infusions of β-endorphin. Infusion of β-endorphin at a rate of 5 μg/min for 5–10 min resulted in very slight changes in spontaneous chemoreceptor discharge and systemic blood pressure (Fig. 3 *A*). Blood gas tensions and pH were unaffected by the infusion.

Evoked responses during infusion of β-endorphin. The increases in chemoreceptor discharge evoked by ACh, CO_2 and NaCN, together with the decrease caused by dopamine, were determined before, during, and after an infusion of β-endorphin at a rate of 5 μg/min. The

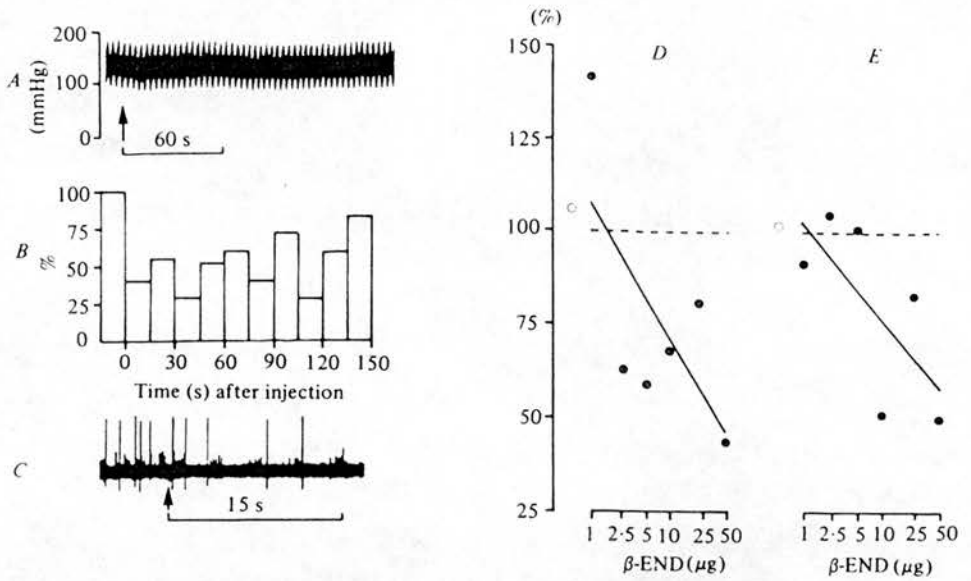

Fig. 1. The left-hand side of the figure shows the effect of injecting (arrow) β-endorphin (50 μg I.C.) on: *A*, systemic arterial blood pressure; *B*, spontaneous chemoreceptor discharge averaged over a 15 s periods and expressed as a percentage (control discharge, 1·5 ct/s = 100%); *C*, the single chemoreceptor unit from which the data in *B*, *D* and *E* were obtained. The right-hand side of the figure shows the discharge averaged over the first (*D*) or second (*E*) minute following β-endorphin injection. Discharge was expressed as a percentage of the pre-injection frequency and plotted against dose of β-endorphin ($\log_{10}$ scale), straight lines being fitted to the responses (●) by the method of least squares. ○, illustrate the effect of injecting the drug vehicle (0·5% bovine serum albumin); the pre-injection (control) discharge frequency (100%) was determined by averaging spontaneous discharge during the 30–60 s immediately preceding each injection, and is represented by the dotted line.

results obtained are summarized in Fig. 4 and it was found that responses to the stimulants were reduced by β-endorphin in the concentration studied, whereas the inhibitory effect of dopamine was potentiated. The influence of β-endorphin on responses evoked by dopamine and, to a lesser extent, NaCN, was still evident 15 min after the infusion had finished.

In a separate experiment the influence of β-endorphin (5 μg/min) on the inhibition evoked by an injection of methionine enkephalin (10 μg I.C.) was studied. The effect of methionine enkephalin was found to be very slightly enhanced. (Discharge, averaged over 150 s after the enkephalin injection and expressed as a percentage of the pre-injection discharge frequency (100%) was 71% before the infusion, 64% during the infusion, and 73% 15 min after the infusion of β-endorphin.)

Vasoactive Intestinal Polypeptide (VIP)

In two experiments the effects of VIP were studied. Only a limited amount of VIP was available, but the results obtained were quite clear.

Injections of VIP. VIP injections (0·05–6·3 μg I.C.) produced the type of effects seen in Fig. 5. Low doses caused a decrease in spontaneous chemoreceptor discharge whereas higher doses increased discharge. The effects were fairly long-lasting, being evident in the second minute following the injection (see Fig. 5*D*). There was a good correlation between dose and response, and higher doses caused a fall in B.P. followed by a rise (Fig. 5*A*).

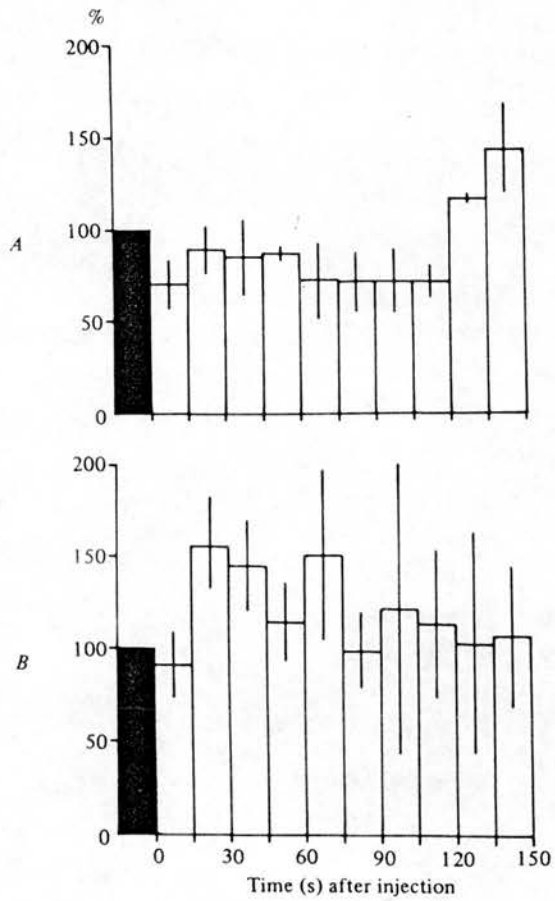

Fig. 2. Chemoreceptor discharge following injection of β-endorphin 10 μg I.C., *A*, before and *B*, 5 min after naloxone (0·4 mg I.C.). Pooled data from three experiments, discharge expressed as a percentage of the pre-injection frequencies and shown as the average $\pm$ S.E. of mean in 15 s intervals during the 150 s post-injection period. The averaged control values were 4·8 $\pm$ 2·0 ct/s before and 2·7 $\pm$ 1·6 ct/s after naloxone.

Infusions of VIP. Infusion of VIP at a rate of 0·5 μg/min for 5–10 min caused a sustained increase in discharge and a fall in blood pressure (see Fig. 3 *B*). The chemoreceptor response shown in Fig. 3 *B*, the biggest observed with the dose of VIP studied, was accompanied by a slight fall in B.P. The control B.P. was rather low, and to preclude the possibility that the increase in chemoreceptor discharge was entirely secondary to the systemic vascular effects of VIP, the infusion was repeated later in the same experiment when mean systemic B.P. was over 100 mmHg and dextran was infused to prevent the fall in B.P. Under these conditions discharge still increased, although the increase was a little less than that obtained at the lower pressure. Measurement of arterial blood gases and pH (see Fig. 3, legend) showed that the increase in chemoreceptor discharge was not secondary to changes in arterial blood gas tensions.

Evoked responses during infusion of VIP. Responses to ACh, CO_2 and NaCN were determined before, during and after infusions of VIP at a rate of 0·5 μg/min. The results

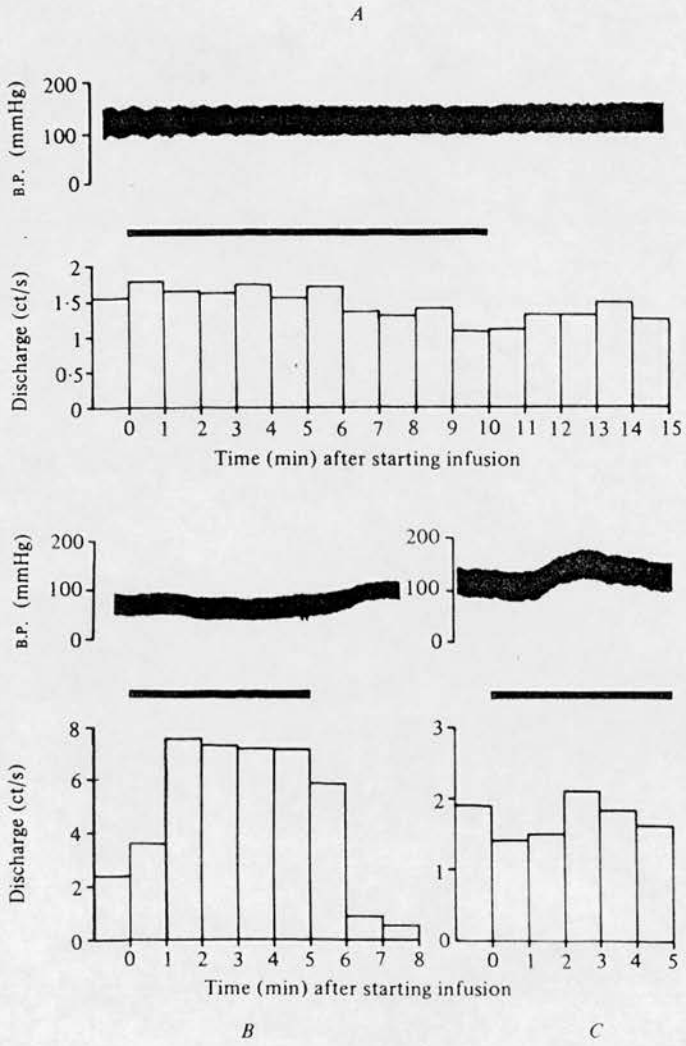

Fig. 3. Effects on spontaneous chemoreceptor discharge of infusing, during the periods represented by the horizontal bars, *A*, β-endorphin 5 μg/min I.C.; *B*, VIP 0·5 μg/min I.C.; *C*, CCK-8 10 μg/min I.C. Discharge (ct/s) was averaged over 60 s intervals, commencing 60 s before starting an infusion, and plotted on the same time scale as the B.P. trace. Just before the end of the VIP infusion in *B*, an arterial blood sample was taken and gave values of pH 7·31, $P_{a.CO_2}$ 107 mmHg, $P_{a.CO_2}$ 34 mmHg compared with pre-infusion sample values of pH 7·28, $P_{a.CO_2}$ 105 mmHg, $P_{a.CO_2}$ 36 mmHg. These results were obtained from three separate experiments, and it should be noted that the catheters were primed with polypeptide solution (i.e. there was no dead-space to be cleared after starting an infusion).

obtained from two experiments were pooled and are summarized in Fig. 4. It was found that responses to the stimulants were reduced by VIP, whereas the inhibitory effect of dopamine was only slightly reduced during the infusion, although there was a further reduction after infusion. Responses to ACh and CO_2 recovered within 15 min, whereas the NaCN and dopamine responses did not.

In one experiment the influence of a VIP infusion (0·5 μg/min) on the inhibition evoked by methionine enkephalin (10 μg I.C.) was studied. Before the VIP infusion chemoreceptor

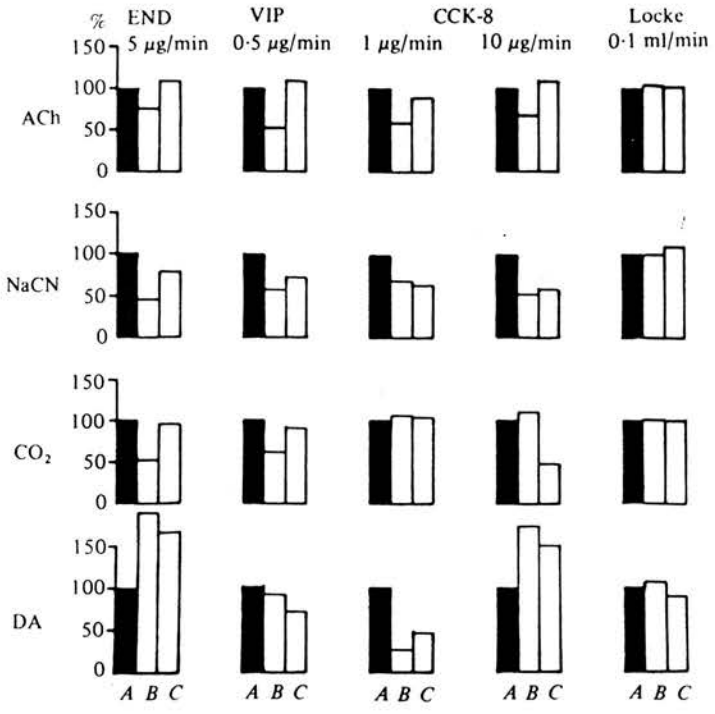

Fig. 4. Responses ($\Delta\Sigma x$) to ACh (50 μg I.C.), NaCN (2·5 μg I.C.), CO_2 (0·3 ml CO_2-saturated Locke solution) and dopamine (DA, 5 μg I.C.) were obtained; *A*, before a 10 min infusion of polypeptide into the second carotid catheter; *B*, during the infusion; *C*, 5–15 min after finishing the infusion, and are shown as percentages (pre-infusion response = 100%). In the case of β-endorphin and CCK-8 data were from single experiments; for VIP and Locke solution the data shown are averages from two experiments. $\Delta\Sigma x = \Sigma x - \bar{x} \cdot t$, where Σx is the number of action potentials counted during the response of duration t s, a 'response' being defined as lasting from the first substantial change from background discharge frequency ($\bar{x}$ *cf*/s) until return to background level.

discharge averaged over the immediate 60 s post-injection period was reduced to 27% of the pre-injection control frequency, and over the second minute it was reduced to 89% of control. Corresponding values during VIP infusion were 46% in the first minute and 100% in the second, meaning that the inhibition caused by methionine enkephalin was reduced during the VIP infusion.

Cholecystokinin octapeptide (CCK-8)

In two experiments the effects of CCK-8 on chemoreceptor activity were examined. Similar effects were obtained in both experiments.

Injections of CCK-8. Injections of CCK-8 (0·1–100 μg I.C.) caused a somewhat variable short-lasting biphasic effect on discharge, generally an initial decrease followed by an increase (see Fig. 6). Higher doses caused a rise in B.P. The quantitative evidence shown in Fig. 6*C* and *D* confirms that responses were somewhat variable and short-lived.

Infusions of CCK-8. In one experiment CCK-8 was infused at a rate of 1 μg/min, in the other the rate was 10 μg/min. There was little effect on spontaneous discharge with either dose (Fig. 3*C*); arterial blood pressure rose.

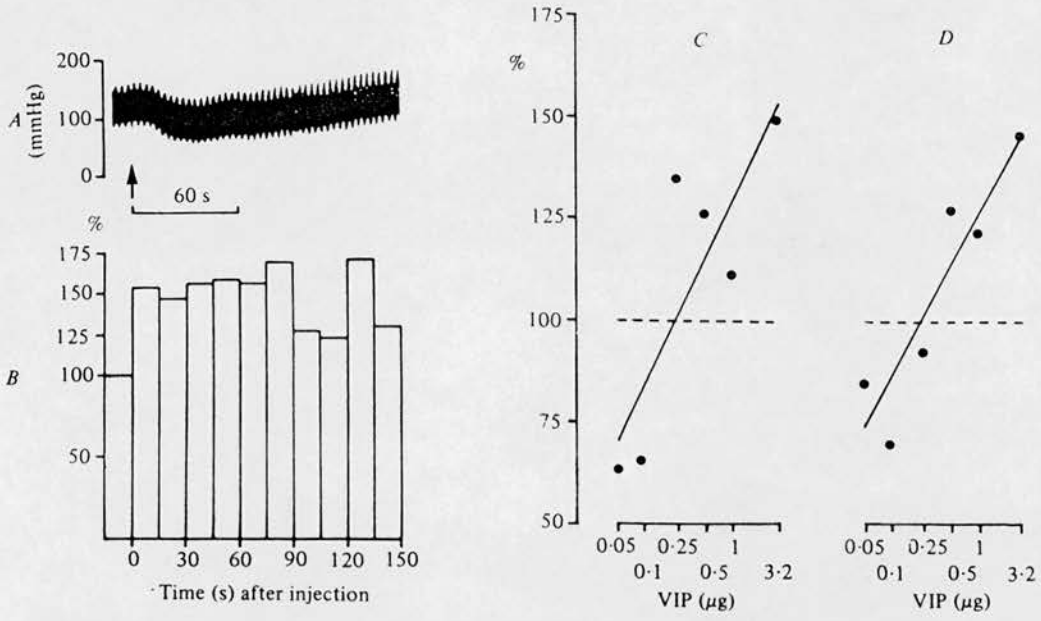

Fig. 5. The left-hand side of the figure shows the effect of injecting VIP (3·2 μg I.C.) on: *A*, systemic arterial blood pressure; *B*, spontaneous chemoreceptor discharge, control frequency (100%) being 5·6 ct/s. The right-hand side of the figure shows data obtained from the same experiment and illustrates discharge averaged over the first (*C*) or second (*D*) minute following VIP injection. Figure details as for Fig. 1.

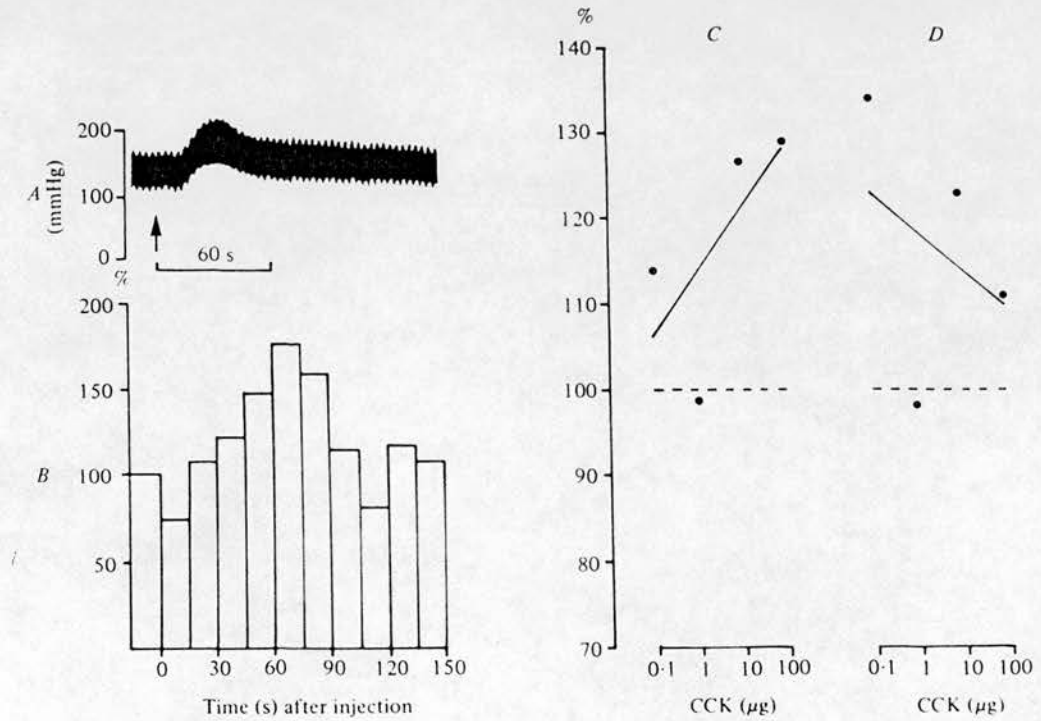

Fig. 6. The left-hand side of the figure shows the effect of injecting CCK-8 (100 μg I.C.) on: *A*, systemic arterial blood pressure; *B*, spontaneous chemoreceptor discharge, control frequency (100%) being 1·9 ct/s. The right-hand side of the figure shows pooled data from two experiments, illustrating discharge averaged over the first (*C*) or second (*D*) minute following CCK-8 injection. Figure details as for Fig. 1.

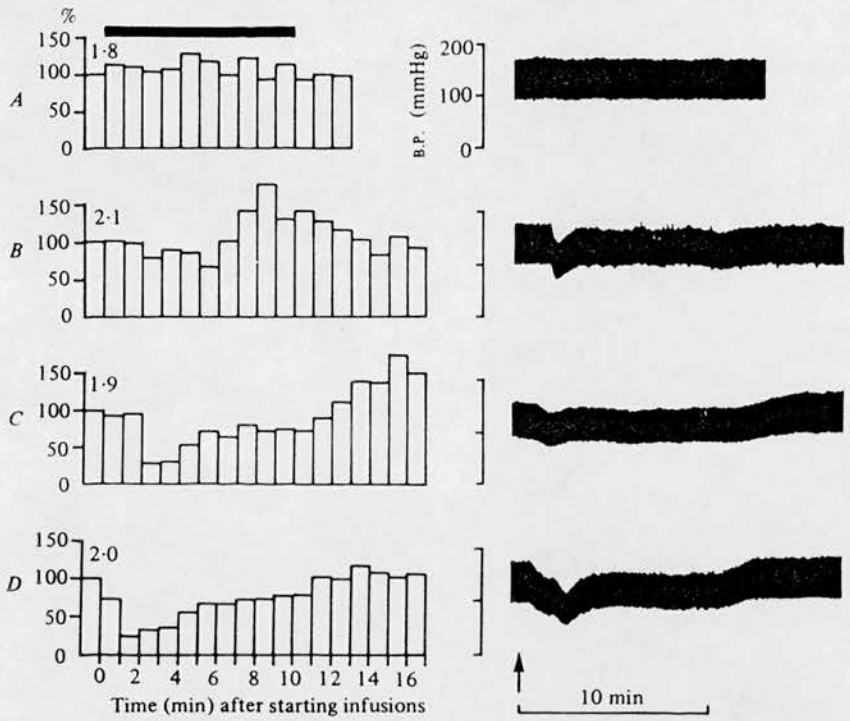

Fig. 7. The left-hand side of the figure shows chemoreceptor discharge (control (100%) values given in ct/s) averaged over 60 s periods after starting intracarotid infusions (horizontal bar) of: *A*, Locke solution via lingual and thyroid catheters; *B*, SP 25 μg/min via the lingual, Locke solution via the thyroid catheter; *C*, methionine enkephalin 10 μg/min via the thyroid, Locke solution via the lingual; *D*, methionine enkephalin 10 μg/min via the thyroid, SP 25 μg/min via the lingual. The accompanying blood pressure records are shown on the right-hand side of the figure, the time scale being common to both sides. The catheter dead-space needed to be cleared before the peptides arrived in the carotid artery, a process which took 1–2 min after starting the infusion, except in *D* where the thyroid catheter was fully primed with methionine enkephalin so that the action of this peptide had begun before SP reached the carotid artery.

Evoked responses during infusion of CCK-8. Responses to ACh, NaCN and DA were reduced during infusion of CCK-8 at a rate of 1 μg/min. The response to CO_2 was unaffected (Fig. 4). Although the ACh effect returned to pre-infusion levels within 15 min of finishing the infusion, responses to NaCN and dopamine remained depressed. In a second experiment, during which CCK-8 was infused at 10 μg/min, responses were somewhat similar except that a sustained potentiation of the dopamine-induced chemoinhibition was observed and there was a decreased response to CO_2 in the post-infusion period, although not during the infusion (Fig. 4).

Substance P and methionine enkephalin

Previous experiments had established that both these polypeptides can influence chemoreceptor discharge (McQueen, 1979; McQueen & Ribeiro, 1980) and the present experiment was undertaken to determine whether any interaction occurs on simultaneous infusion of both peptides close-arterial to the carotid body. The results obtained are shown in Fig. 7. Locke solution caused a very slight increase in spontaneous chemoreceptor discharge, SP had a biphasic action (decrease followed by increase) and methionine enkephalin reduced

discharge, an effect which was most pronounced in the early part of the response; when the methionine enkephalin infusion was stopped discharge increased above pre-infusion control levels. The delayed increase in discharge seen with SP was accompanied by a rise in end-tidal CO_2; there was no change in end-tidal CO_2 during or after methionine enkephalin infusion.

SP and methionine enkephalin were infused concurrently in equimolar concentrations, it being so arranged that methionine enkephalin began to act before SP reached the carotid artery (see Fig. 7). The pattern of response was very similar to that obtained with methionine enkephalin alone, except that no post-infusion overshoot occurred. There was a rise in end-tidal CO_2 towards the end of the infusion. Both peptides caused hypotension, and when given together the overall effect on B.P. was approximately additive.

DISCUSSION

The present study showed that the polypeptides investigated can modify carotid chemoreceptor activity in the cat. Spontaneous chemoreceptor discharge was decreased by intracarotid injection of β-endorphin and by low doses of VIP, whereas it was increased by CCK-8 and higher doses of VIP. The chemo-excitatory effect of VIP has been reported by Fitzgerald, Raff, Garger, Fechter, Anand & Said (1979). The effects of these peptides on chemoreceptor discharge may not seem very impressive when compared with those of ACh, NaCN, CO_2 or dopamine, but it has to be remembered that the polypeptides have a relatively high molecular mass so that, for example, whereas the excitatory response to ACh was elicited by 180 nmol (50 μg), that to VIP involved only 1 nmol (3·2 μg). Furthermore, although the peptide effects were generally less intense they tended to be longer lasting than those associated with classical neurotransmitters. The prolonged action may be related to various factors, such as molecular size, tissue penetration, dissociation from receptors, activation of 'second messengers', or to peptide inactivation, and needs to be further investigated.

Responses evoked by ACh, NaCN, CO_2 and dopamine during polypeptide infusion, when peptide levels in the carotid body were assumed to be relatively steady, showed that the excitatory action of ACh and NaCN was reduced by all three peptides studied, and that caused by CO_2 was reduced by both β-endorphin and VIP, but unaffected by CCK-8. Dopamine-induced chemoinhibition was potentiated by β-endorphin, largely unaltered by VIP and reduced by low concentrations of CCK-8 but potentiated by higher concentrations. Lack of sufficient peptide precluded full dose response studies (e.g. McQueen, 1977), but although the results obtained with single submaximal doses may be less reliable, they do indicate that polypeptides can influence evoked response when infused in fairly low concentrations. It is particularly intriguing that polypeptides are sometimes stored with putative neurotransmitters (see Pearse, 1969), for example VIP with ACh (Lundberg, Hökfelt, Schultzberg, Uvnäs-Wallensten, Köhler & Said, 1979), enkephalin with noradrenaline (Schultzberg, Lundberg, Hökfelt, Terenius, Brandt, Elde & Goldstein, 1978), SP with 5-hydroxytryptamine (Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow & Goldstein, 1978) and CCK-8 with dopamine (Hökfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). All these putative neurotransmitters are present in the carotid body (see Biscoe, 1972) and so, it appears, are several of the polypeptides. It will be most interesting to determine whether similar dual localization of peptide and amine occurs in the carotid body and whether the distribution of peptides and peptides/amines within the carotid body is homogeneous, or whether different regions or nerve fibres (A

and C, afferent, efferent) have different representation. It may transpire that all the different substances in the carotid body, although activating different receptors, eventually influence a common mechanism (e.g. adenylate cyclase) within the carotid body.

The action of β-endorphin on the chemoreceptors was similar to that of morphine (McQueen & Ribeiro, 1980), there being a tendency for inhibitory responses to be biphasic, and greatly reduced by naloxone (0·4 mg I.C.). Following naloxone an overall increase in discharge occurs (Fig. 2). These similarities might mean that both substances affect the same receptors, with β-endorphin being about twenty times more potent than morphine on a molar basis. The enkephalins, in contrast, cause a much greater chemoinhibition which is not so readily prevented by naloxone, and also have greater hypotensive effects (McQueen & Ribeiro, 1980, 1981). Thus, the chemoinhibition caused by β-endorphin appears to result from actions on naloxone-sensitive opiate receptors, whereas the slight chemoexcitation seen after naloxone does not. The latter effect may result from actions at opiate receptors which are insensitive to naloxone in the doses used, or to direct or indirect actions on non-opiate receptors.

The polypeptides influenced arterial blood pressure and this confirmed that, in the doses studied, they were biologically active. The possibility that the effect of polypeptides on chemoreceptor activity was secondary to vascular changes either within the carotid body, or systemically, needs to be considered. The rapid onset of the peptide effect (within 1–2 s of injection), the fact that effects on chemoreceptor discharge occurred before changes in blood pressure were seen and persisted when such changes were prevented, and the knowledge that chemoreceptor discharge appears to be largely independent of blood pressure over the physiological range (Hornbein, Griffo & Roos, 1961; Biscoe, Purves & Sampson, 1970; Acker, Keller, Lübbers, Bingman, Schulze & Caspers, 1973) and probably carotid body blood flow (Acker & Lübbers, 1977) lead us to consider it unlikely that vascular effects were responsible for the greater part of the responses observed.

The fact that most of the peptides affect the vasculature could be taken to imply that the role of endogenous carotid body peptides is to modify blood flow. However, it is not known whether endogenous polypeptides do in fact modify carotid body blood flow when released, and many established neurotransmitters, such as ACh, noradrenaline and dopamine, have marked vascular effects when injected. This, albeit together with other evidence, has not prevented these latter substances, when present in the carotid body (e.g. see Biscoe, 1972), from being considered as putative chemoreceptor neurotransmitters, so by the same token the vascular effects caused by exogenous polypeptides should not exclude them from consideration as putative neurotransmitters when found to be present in the carotid body.

During the present study we investigated whether any interaction occurred in the carotid body between SP and methionine enkephalin. These two peptides, or closely related material, are present in the cat carotid body (see Introduction) and SP has been claimed to be a neurotransmitter at the central nerve terminals of baro- and chemoreceptor afferent fibres of cats (Gillis, Helke, Hamilton, Norman & Jacobowitz, 1980) and rats (Helke, O'Donohue & Jacobowitz, 1980; Jacobowitz & Helke, 1980). It is also known that methionine enkephalin can inhibit the release of SP from cultured sensory neurones (Mudge, Leeman & Fishbach, 1979) and probably in the c.n.s. (Jessel & Iversen, 1977). Our results showed that SP was unable to overcome the chemoinhibitory effect of methionine enkephalin, something it might have been expected to do if release of endogenous SP is crucial for chemoexcitation, and can be prevented by methionine enkephalin. However, much may depend on the type of fibre being recorded and the background level of activity;

SP is particularly associated with C fibres (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygårds & Pernow, 1977) and it could be that A fibres were being recorded which are, perhaps, affected more by methionine enkephalin than by SP. In the absence of conduction velocity measurements this must remain speculation, and also we don't know whether the concentration of injected SP in the carotid body was physiological. As far as the other polypeptides were concerned, the inhibition evoked by methionine enkephalin was slightly potentiated by β-endorphin but reduced by VIP.

Evidence from other parts of the nervous system suggests that some polypeptides may function as neurotransmitters (see Hökfelt *et al.* 1980), but the question of what physiological role the polypeptides play in the nervous system is still very much a matter for debate (see Bishop & Polak, 1978; Guillemin, 1978; Hökfelt *et al.* 1980; Snyder, 1980). As far as the carotid body is concerned, various exogenous polypeptides affect chemoreceptor activity and several of these are known to be present in the structure. However, on its own this information does not enable us to reach a conclusion regarding the role of the peptides in the carotid body. They may function as neurotransmitters, neuromodulators, co-transmitters affecting receptor sensitivity, neurohormones, trophic factors, or as agents modifying the vasculature, and might be influenced by each other, by carotid body amines, by circulating hormones (e.g. ACTH, β-endorphin) or by other substances.

In conclusion, further studies are needed in order to determine the role of polypeptides in carotid body chemoreception, and these will involve the use of drugs which can selectively affect the peptidergic system (e.g. by destruction of nerve cells, blockade of receptors, inhibition of enzymatic destruction, interference with biosynthesis) and also the correlation of carotid body peptide release with chemoreceptor activity under various physiological conditions. It will also be necessary to establish whether the polypeptides which appear to be present in the carotid body are the endogenous physiologically active entities, or whether they are converted to or derived from more active forms. Most of these ideals seem unattainable at present because of lack of suitable drugs or techniques, but this situation may change rapidly.

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The inhibitory effect of opiates on carotid body chemoreceptors is not mediated by adenosine

J. A. Ribeiro, D. S. McQueen

Department of Pharmacology, University of Edinburgh, EH8 9JZ, Scotland

It has been suggested that the inhibition of neurotransmitter release produced by opiates may be mediated by the initial release of adenosine [5]. The idea was advanced on the grounds that adenosine mimicks the effect of morphine, and theophylline antagonizes the actions of both adenosine and morphine. In the cat carotid body, adenosine stimulates [4] and the opiates (morphine, methionine- or leucine-enkephalin) depress [2, 3] spontaneous chemoreceptor activity. The present work was undertaken to investigate the effect on the chemoreceptors of theophylline and its influence on both adenosine and opiates.

Experiments were performed on cats anaesthetized with pento-barbitone (42 mg kg, i.p. supplemented every 1-2 hr). The animals were artificially ventilated with air and paralysed with gallamine (3 mg kg, i.v.). Chemoreceptor activity was recorded from the peripheral end of a sectioned carotid sinus nerve [1]; the ganglioglomerular (sympathetic) nerves were usually cut. Drugs were dissolved in Locke solution and injected into the ipsilateral common carotid artery (i.c.) over a 2 sec. period.

Theophylline (100-200 μg, i.c.) had little or no effect on spontaneous chemoreceptor discharge but a higher dose (1 mg, i.c.) depressed discharge, an effect which lasted for about 30 s. The excitatory action of adenosine (1-100 μg, i.c.) was potentiated after theophylline (1 mg, i.c.) whereas the inhibitory action of methionine-enkephalin (10 μg, i.c.) was unaffected and that of morphine (10-100 μg, i.c.) slightly reduced ($p > 0.05$). Naloxone (400 μg, i.c.) antagonized the chemodepressant effects of morphine and methionine-enkephalin, but did not alter the chemoexcitatory effect of adenosine.

Since adenosine does not mimick the effect of opiates on the chemoreceptors, and the actions of the opiates were not prevented by theophylline, it appears unlikely that adenosine mediates the chemoinhibitory action of opiates in the cat carotid body.

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Antihypertenseurs et prévention du développement de l'hypertension génétique chez le SHR

C. Richer, J. L. Freslon, J. F. Giudicelli

Département de Pharmacologie, Faculté de Médecine Paris-Sud, 63, rue Gabriel-Péri, F 94270 Le Kremlin-Bicêtre

Si la plupart des antihypertenseurs sont actifs vis-à-vis de l'hypertension génétique du SHR adulte, les inhibiteurs β-adrénergiques constituent une exception de taille. Aussi, ces dernières années une autre approche du problème a été envisagée, consistant à rechercher dans quelle mesure les antihypertenseurs administrés de manière chronique chez le

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OPIOID PEPTIDE INTERACTIONS WITH RESPIRATORY AND CIRCULATORY SYSTEMS

D S McQUEEN BPharm PhD

Department of Pharmacology
University of Edinburgh Medical School

The brain-stem contains neurones which form part of the systems involved in controlling respiration and blood pressure. Opioid peptides are present in this region of the brain, and recent evidence implicating these substances in central respiratory and cardiovascular regulation will be reviewed in this paper.

1 The Brain-Stem, Opioid Receptors and Opioid Peptides

Cranial nerves IX and X convey information from peripheral sensory receptors to the brain-stem—arterial chemoreceptors and baroreceptors provide information to the central nervous system (c.n.s.) concerning blood gas tensions and blood pressure/pulse frequency respectively. A high proportion of these primary afferent nerve fibres have their first synapse in the nucleus tractus solitarii (NTS) of the medulla oblongata (see Palkovits & Záborsky, 1977).

It has been known for some time that certain regions of the brain-stem, particularly the ventrolateral surface, are pharmacologically sensitive, or "chemosensitive", and can give rise to respiratory and/or cardiovascular changes when affected by various substances, including the opiate drug morphine (Loeschcke & Koepchen, 1958; Feldberg, 1976). The existence of specific opioid receptors was demonstrated pharmacologically by using stereoisomers of opiates and, more recently, naloxone which antagonizes central actions of morphine and is regarded by many as being a specific antagonist for opioid receptors. More recently, binding sites for opiates have been localized in parts of the brain-stem involved in respiratory and cardiovascular control (see Atweh & Kuhar, 1977, 1983), as have enkephalinergic neurones and their terminals (Hökfelt *et al.* 1977), especially in the NTS (Uhl *et al.* 1979).

2 Respiratory System

It is well known that therapeutic doses of morphine depress ventilation, and this generally undesirable effect can be attributed to a reduction in the sensitivity of neurones in the brain-stem to carbon dioxide (Flórez *et al.* 1968). Iontophoresis of morphine or [Met]enkephalin reduces the peak discharge frequency without affecting basal discharge of respiration-related units in the tractus solitarius, nucleus ambiguus and nucleus parabrachalis medialis of the cat brain (Denavit-Saubie *et al.* 1978), and histochemical techniques have shown that opioid receptors and enkephalin-like material are present in these regions (see Frederickson, 1977).

The ventral surface of the medulla oblongata is very sensitive to the respiratory-depressant effects of opiates and opioids (Flórez & Mediavilla, 1977; Moss & Friedman, 1978; Flórez *et al.* 1980), and chemosensitive structures that monitor the extracellular pH and P_{CO_2} of brain fluid are located 200–300μm beneath this area (Mitchell *et al.* 1963). These chemosensitive zones are more superficially sited than are the classical respiratory "centres", which are situated close to the floor of the fourth ventricle. It seems probable that opioids depress the responsiveness of the chemosensors to carbon dioxide (Zobrist *et al.* 1981) and may play a physiological role in the control of respiration (Pokorski *et al.* 1981).

The peripheral chemosensors also contain enkephalins (Wharton *et al.* 1980), and in the case of the cat carotid body [Met]-enkephalin-like material appears to coexist with catecholamines in glomus cells, possibly within the same vesicles (Hansen *et al.* 1982). Chemosensory discharge is depressed by exogenous enkephalins (see Fig. 1), but morphine and β-endorphin are much less potent than are [Met]- and [Leu]-enkephalin in depressing carotid chemoreceptor activity in cats (McQueen & Ribeiro, 1980, 1981). Low doses of naxolone can abolish the depressant effects of morphine and β-endorphin on discharge, but higher doses are needed to produce a comparable reduction in the responses to [Leu]- and [Met]-enkephalin (Fig. 2). This could be interpreted as evidence for two types of opioid receptor in the carotid body, namely μ (morphine) and δ (enkephalin) subtypes, the latter being more potent in evoking depression of chemoreceptor activity.

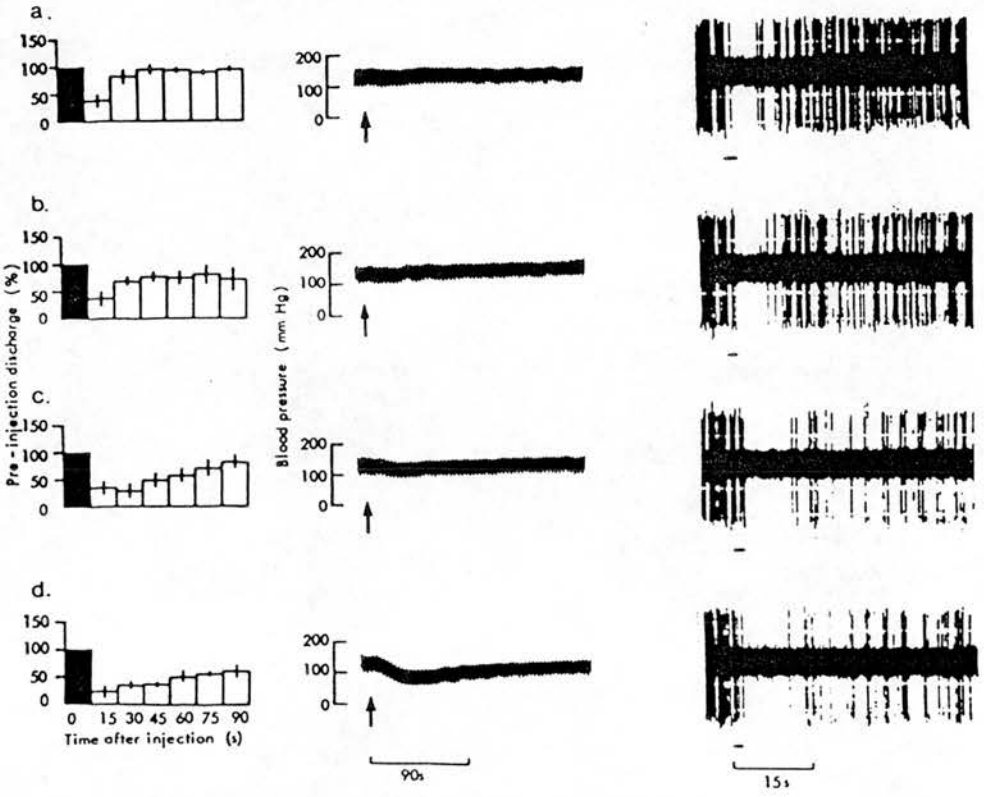
Since morphine's depressant effect on peripheral arterial chemoreceptors is relatively slight, it follows that the greater part of respiratory depression associated with this opiate results from actions in the c.n.s. In contrast, [Met]enkephalin depresses peripheral as well as central chemosensors. The physiological function of opioid receptors and opioids in the carotid body, and their relationship to other substances, such as catecholamines, 5-hydroxytryptamine, vasoactive intestinal polypeptide, substance P and acetylcholine, which are also present in these sensory organs, remains to be established. Naloxone itself causes a slight increase in chemosensory discharge under normoxic and normocapnic conditions (McQueen & Ribeiro, 1980), which could mean that endogenous opioids act within the carotid body to reduce peripheral chemoreceptor drive to respiration (Pokorski & Lahiri, 1981). This may have very important consequences because the c.n.s. has no intrinsic mechanism for detecting hypoxia and initiating respiratory changes, being reliant on the reflex stimulation of respiration which results from activation of arterial chemoreceptors under hypoxic conditions. Excessive opioid release within the carotid body, or high levels of circulating opioids, could impair or blunt the reflex respiratory response to hypoxia.

a Physiological Role

The presence of opioid peptides and opioid receptors in the brain-stem and the potent respiratory-depressant effects of opioid

FIG. 1. Effect of [Met]enkephalin on chemoreceptor discharge

(Reprinted, with permission, from McQueen, 1981)



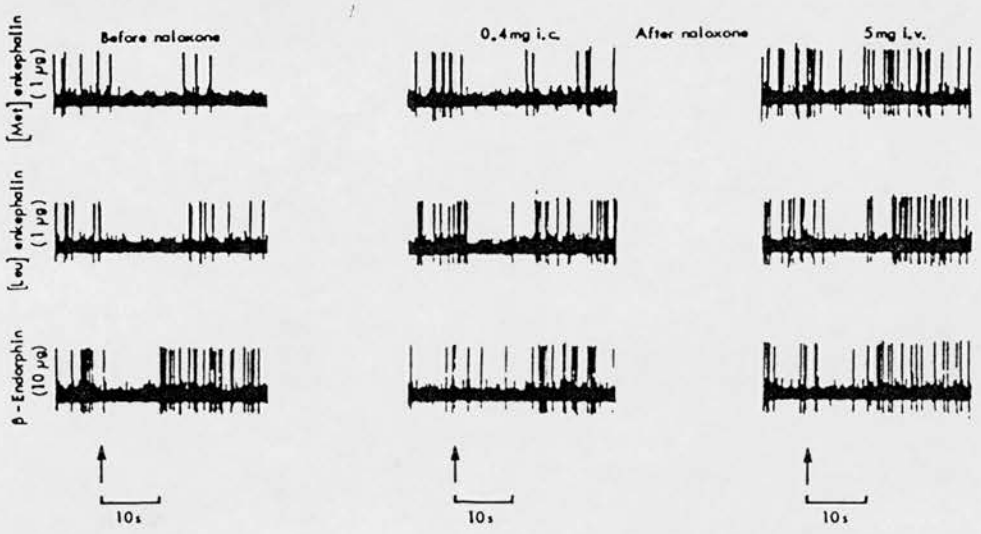
a: 0.1μg [Met]enkephalin b: 1.0μg [Met]enkephalin c: 10μg [Met]enkephalin d: 100μg [Met]enkephalin
[Met]enkephalin injected into the carotid artery causes a dose-related depression of chemosensory discharge in cats. The left-hand panel shows data pooled from four cats in which discharge was averaged over 15 s periods following injection of [Met]enkephalin and expressed as the mean percentage (± S.E.M.) of the pre-injection discharge (black rectangle = 100%). The central panel illustrates the effect of the opioid peptide (injected at arrow) on femoral arterial blood pressure in one experiment, and the right-hand panel of neurograms shows the discharge of a single chemoreceptor unit recorded in the same experiment illustrating the depressant effect of [Met]enkephalin (injected at the marker)

peptides and opiates applied locally to this area led to the suggestion that these substances may be involved in regulating respiration by exerting a tonic inhibition at one or more sites (e.g. Moss & Scarpelli, 1981). If this is the case, it would be expected that the opioid receptor antagonist naloxone would increase ventilation, and several studies have shown that this does indeed occur (Stephen *et al.* 1976; Moss & Friedman, 1978; Lawson *et al.* 1979; Moss & Scarpelli, 1979). In contrast, others report that naloxone has no effect on ventilation (Chernick & Russell, 1978; Willer *et al.* 1979; Fleetham *et al.* 1980), although it does reduce morphine-induced respiratory depression (Kaufman *et al.* 1981). Various species, including man, have been used in these studies and it could be argued that differences in species and doses of naloxone used account for the discrepant results. However, it seems that the conditions under which the experiments are performed determine whether or not naloxone affects ventilation. For example, the respiratory-stimulating effect of naloxone, in the absence of hypoxaemia, is age dependent, being virtually abolished four to five days after birth in rabbits (Hazinski *et al.* 1981). When stressed with hypoxaemia adequate to produce respiratory depression, maturing rabbits as well as very young animals show a marked increase in respiration following naloxone administration (Stephen *et al.* 1976; Chernick *et al.* 1980; Grunstein *et al.* 1981). The observations with naloxone provide indirect evidence that during acute hypoxaemia endogenous opioid peptides are released and may act as respiratory depressants.

The ventilatory response to moderate hypoxia in the human neonate is, unlike that of the adult, biphasic. Initially ventilation

FIG. 2. Responses of a single chemoreceptor unit to intracarotid (i.c.) injections (arrows) of [Met]- and [Leu]-enkephalin and β-endorphin before and after an initial intracarotid dose, followed by an intravenous (i.v.) dose, of naloxone in an anaesthetized cat

(McQueen, unpublished work)



increases, but instead of being sustained, as in the adult, a decrease in ventilation occurs which may persist for a long time, despite return to normoxic conditions (Cross *et al.* 1954). Might the respiratory depression be caused by opioids? In this connection it is interesting that levels of β-endorphin-like material in umbilical cords are increased in hypoxaemic infants (Wardlaw *et al.* 1979). The respiratory responses of young animals to naloxone may represent removal of an inhibitory influence of opioid peptides on neuronal pathways involved in regulating respiration during early postnatal life, and raises the possibility that certain abnormalities in human neonates (e.g. periodic breathing and apnoea) and older infants (e.g. sudden infant death syndrome—cot death) may be related to actions of opioids. The role of opioid peptides in respiratory control in the fetus needs more detailed investigation, but initial reports suggest that naloxone does not increase breathing activity during non-rapid-eye-movement (REM) sleep, and the apnoea of prematurity is not ameliorated or abolished by naloxone (see Chernick, 1981).

Naloxone increases respiration in anaesthetized animals (e.g. Lawson *et al.* 1979), but these observations cannot be extrapolated to the normal conscious animal because, apart from the direct effects of the anaesthetic, the animals were exposed to the stresses associated with anaesthesia and surgery. Stress leads to increased blood levels of β-endorphin (Rossier *et al.* 1977), and this peptide can enter the c.n.s. (Rapoport *et al.* 1980). When experiments are performed on unstressed conscious humans, naloxone does not increase spontaneous respiration (Willer *et al.* 1979) or respiratory responses to either hypoxia or hypercapnia, even following massive doses (50mg intravenously (i.v.): Fleetham *et al.* 1980) of the antagonist, several orders higher than needed for abolishing the respiratory-depressant action of morphine in man. Incidentally, the administration of such high doses of naloxone to anaesthetized or stressed patients is potentially dangerous for reasons which are not clear, but may involve massive increases in sympathetic tone (Andree, 1980).

Naloxone improves respiration in rats following electroconvulsive shock (Holaday *et al.* 1978) or shock associated with spinal cord transection (Holaday & Faden, 1980). The action of the opiate antagonist is mediated centrally and is presumed to result from a blockage of endogenous opioid peptide action.

Some studies have been performed on patients suffering from chronic obstructive pulmonary disease (c.o.p.d.) to determine whether endogenous opioids, released in response to the stress of chronic dyspnoea, might cause respiratory depression. Santiago *et al.* (1981) found naloxone (2mg i.v.) restored flow-sensitive load compensation without affecting respiratory sensitivity to CO_2, whereas Butland *et al.* (1981) were unable to demonstrate any ventilatory effect in their patients who received 0.4–4mg naloxone. Obtaining meaningful data from respiratory experiments in conscious man, whether patients or healthy volunteers, is notoriously difficult, and adequate controls are needed.

b *Interactions with Putative Transmitters*

Morphine can affect the levels and release of substances such as acetylcholine, 5-hydroxytryptamine and noradrenaline in the medulla oblongata, and morphine and opioid peptides may induce respiratory depression by affecting one or more of these putative transmitters (e.g. see Ahtee & Attila, 1980; Meldrum & Isom, 1981). Much more work needs to be done to see how opioid peptides interact with neurones and neurotransmitters involved in controlling respiration, but it would not be surprising to find that

the release of one or more transmitters is affected by opioids. The question to be addressed is whether such effects have any physiological or pathophysiological significance for respiratory control.

3 Cardiovascular System

a *Opiates*

Morphine can affect systemic arterial blood pressure and heart rate, the greater part of the effects resulting from actions on opioid receptors in the c.n.s. Studies in anaesthetized artificially ventilated cats have shown that morphine produces quite different cardiovascular effects when injected into a lateral ventricle or into the cisterna magna: pronounced tachycardia with a short-lasting rise in blood pressure due to sympathetic stimulation on intraventricular injection, and bradycardia and a gradual long-lasting fall in pressure due to removal of sympathetic tone to heart and vessels on intracisternal injection, the latter effect being similar to that evoked by morphine (0.5–1 mg/kg) injected subcutaneously (Feldberg & Wei, 1977). The bradycardia and prolonged fall in blood pressure on intracisternal injection resulted from an action on structures reached from the subarachnoid space because, when administered in this way, morphine does not enter the cerebral ventricles. The structures affected by morphine are considered by Feldberg & Wei (1978) to be at the dorsal surface of the medulla, since the depressor effects were obtained when the drug was applied topically to the dorsal but not to the ventral surface of the brain. Morphine acts in the same region at the dorsal surface when injected subcutaneously because the fall in heart rate and blood pressure was prevented or restored by topical application of naloxone (about 10μg) to this region. Feldberg & Wei suggest that the commissural nucleus of the tractus solitarius may be the structure on which morphine is acting to inhibit sympathetic tone to blood vessels and the heart on subcutaneous or intracisternal injection.

b *Opioid Peptides*

Application of [Met]enkephalin to the ventral surface of the brain-stem causes a reduction in blood pressure, as well as respiratory depression in cats, and these effects can be prevented by naloxone (Flórez & Mediavilla, 1977). Injections of [Leu]- and [Met]-enkephalin into the brain produce either hypertension or hypotension according to where the injection is made, [Leu]enkephalin tending to cause hypertension in rats and cats (Schaz *et al.* 1980). This has led to the suggestion that there are at least two types of opioid receptor within the medulla oblongata (Fuxe *et al.* 1979): one type is relatively resistant to naloxone and is involved with vasopressor responses, whereas the other type is readily antagonized by naloxone and is involved with vasodepressor responses. It is interesting to note that vasopressin appears to be necessary for the [Leu]enkephalin pressor effects, because Brattleboro rats, which lack vasopressin, do not show pressor responses to this opioid. Bisset *et al.* (1978) have described the release of vasopressin by enkephalins, and this may account for the delayed onset of the pressor response to [Leu]enkephalin.

c *Opioid Peptide Involvement in Cardiovascular Regulation*

The existence of opioid-containing nerves and opioid receptors in brain-stem regions that are involved in cardiovascular regulation, together with the effects of exogenous opioids and opiates on blood pressure, has led to suggestions that opioid peptides are involved in the physiological control of the cardiovascular system. There is also evidence that they interact with both the sympathetic and parasympathetic divisions of the autonomic nervous system, which are involved in cardiovascular regulation (Laubie & Schmidtt, 1981).

Morphine-like drugs cause a centrally mediated hypotension and bradycardia, an effect which is very similar to that evoked by activating arterial baroreceptors and raises the question of whether opioids are involved in modifying activity of the baroreceptor reflex pathway. In fact it appears that opioids act at μ-receptors to attenuate the baroreceptor reflex in conscious rabbits (Petty & Reid, 1981), an action which is antagonized by naloxone. Naloxone, given on its own, reportedly increases baroreflex activity, but the evidence is not particularly convincing. Kumazawa *et al.* (1981) found naloxone caused a slight rise in blood pressure in anaesthetized dogs that had undergone bilateral vagotomy and sectioning of the carotid sinus nerves, and this action of naloxone (0.1–2 mg/kg i.v.) was not dose dependent. The state of the experimental animal seems to determine whether naloxone elicits a pressor response (see also Dashwood & Feldberg, 1980), and the antagonist appears to have no effect on heart rate (Willer *et al.* 1979) or blood pressure in normal man.

Naloxone can reverse the hypotensive effect of clonidine (Farsang *et al.* 1980), and Kunos *et al.* (1981) have demonstrated that clonidine releases a β-endorphin-like peptide from slices of brain-stem. The evidence is compatible with a depressor role for β-endorphin (see also Petty *et al.* 1981). However, in order to implicate the opioid peptides in central cardiovascular control it will be necessary to show changes in their output within discrete brain regions under varying physiological conditions.

d *Shock*

Naloxone acts at central opioid receptors to reverse the hypotension, hypothermia and hypoventilation of spinal shock in rats (Holaday & Faden, 1980). Hypotension associated with hypovolaemic shock in dogs can also be reversed by naloxone (Vargish *et al.* 1980), and the opiate antagonist has little effect on cardiovascular haemodynamics in normal animals. The stress associated with bleeding anaesthetized dogs to a mean blood pressure of 6 kPa causes release of β-endorphin from the pituitary, and it is possible that this contributes to the shock state by affecting naloxone-sensitive opioid receptors. Release of opioid peptides has been implicated in the pathophysiology of endotoxic (Holaday & Faden, 1978) and hypovolaemic (Faden & Holaday, 1979) shock in rats. The ability of intraventricularly administered (−)-naloxone to reverse the hypotension of shock, but not (+)-naloxone which has no appreciable effects as an opioid receptor antagonist, indicates that the effects of naloxone in shock are mediated through opioid receptors in the c.n.s. The receptors appear to be associated with supraspinal parasympathetic pathways because transection of the cord interrupts communication between higher brain centres. The isolation of sympathetic neurones from higher control leaves the parasympathetic nervous system as the primary means for neural regulation of cardiovascular function. Holaday & Faden (1980) have drawn attention to the possible therapeutic benefit of narcotic antagonists in treating spinal cord injury by improving spinal cord blood flow and thereby reducing spinal ischaemia.

e *Hypertension*

Levels of enkephalins in the neurohypophysis are raised in spontaneously hypertensive rats, but not in animals with desoxycorticosterone acetate/salt-induced hypertension (Morris *et al.*

1981). The changes in level of enkephalin could be due to decreased release or increased synthesis, and the relevance of the observation to genetic hypertension remains to be elucidated.

f *Interactions with Putative Transmitters*

Costa *et al.* (1978) have reviewed the interactions that can occur between enkephalinergic and other neuronal systems. Noradrenaline and adrenaline are considered to be important neurotransmitters for cardiovascular control, noradrenaline causing vasopressor and adrenaline vasodepressor responses (see Fuxe *et al.* 1979), and noradrenaline- and adrenaline-containing neurones are present in the NTS. Terminals of noradrenergic neurones have specific opioid receptors (Starke, 1977), and activation of these presynaptic receptors by opioids could lead to a reduction in the amount of transmitter released by each impulse. There is some evidence for interaction between opioid peptides and catecholamines in central cardiovascular regulation (Fuxe *et al.* 1980), and morphine and opioid peptides can also modify brain monoamine synthesis.

Since the precise role of adrenaline, noradrenaline, dopamine, acetylcholine and 5-hydroxytryptamine in central cardiovascular control remains to be established, it is difficult to interpret meaningfully the preliminary results that have been obtained from experiments aimed at examining the interactions between these putative transmitters and the opioid peptides. Furthermore, interactions between the opioids and other peptides (e.g. substance P, which is claimed to be a transmitter at the central terminals of baro- and chemo-receptor afferent nerve fibres in the NTS: Gillis *et al.* 1980) may also be involved in the central regulation of blood pressure (Fuxe *et al.* 1980).

4 Summary

Experiments in animals have shown fairly clearly that exogenous opioid peptides can alter respiration and blood pressure by affecting the central systems involved in respiratory and cardiovascular control, although the precise mechanisms remain to be established and actions may take place at more than one site. The interactions between various putative transmitters and opioid peptides, some of which appear to coexist in certain neurones, and may be coreleased, are being studied.

Changes seen following administration of naloxone are generally assumed to result from blockade of opioid receptors and are taken as providing indirect evidence for opioid involvement. However, some caution is needed lest naloxone should prove not to be a selective antagonist for opioid receptors (Sawynok *et al.* 1979) or be incapable, in the doses commonly used, of antagonizing the actions of particular opioids in the brain (i.e. there may be naloxone-insensitive opioid receptors: see Paterson *et al.* 1983). In order to demonstrate involvement of an opioid in a system under physiological or pathophysiological conditions it would be best to obtain evidence (e.g. by measuring opioid release, studying the effects of inhibiting opioid inactivation, using other antagonists and selective agonists) in addition to that provided by naloxone.

The available evidence, based on the interpretation of results from studies with naloxone, does not support involvement of opioid peptides in normal respiratory and cardiovascular control in man. However, in stressful conditions (e.g. anaesthesia, surgery, pain, hypoxia, shock, the neonatal state, and certain pathophysiological disorders) there is evidence from studies in animals and in man that opioid peptides do affect the central regulation of the respiratory and cardiovascular systems, and this has important clinical implications.

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Changes in responses of cat carotid body chemoreceptors to 5-HT after administration of the antagonist MDL 72222

By G. C. Kirby and D. S. McQueen. *Department of Pharmacology, University of Edinburgh Medical School, Edinburgh EH8 9JZ*

The physiological role of the 5-hydroxytryptamine (5-HT) that is present in the cat carotid body (Chiocchio, Biscardi & Tramezzani, 1967) has yet to be established. Nishi (1975) found that intracarotid (I.C.) injection of 5-HT in cats caused a transient increase in carotid chemoreceptor discharge that was followed by a period of chemodepression. These responses were unaffected by the putative 5-HT antagonists LSD, gramine and methysergide.

In the present study we have investigated the effects of MDL 72222 (a new compound which appears to be a selective antagonist at neuronal 5-HT receptors – Fozard, 1983) on the responses of carotid-body chemoreceptors to 5-HT, dopamine and to changes in P_{a,O_2}. Experiments were performed on cats anaesthetized with α-chloralose (70 mg kg^{-1} I.V.) artificially ventilated and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemoreceptor activity was recorded from the peripheral end of a carotid sinus nerve, and drugs were injected into the common carotid artery, as previously described (Docherty & McQueen, 1978).

Injection of 5-HT (0·1–100 μg I.C.) caused a dose-dependent depression of 'spontaneous' chemoreceptor discharge which was followed by a delayed excitation of variable duration. Only occasionally was the chemodepression preceded by a transient burst of activity, in agreement with the findings of Black, Comroe & Jacobs (1972). After MDL 72222 the dose–response curve for 5-HT-evoked chemodepression was shifted to the right, and following the higher dose of antagonist 5-HT caused a prolonged dose-dependent chemo-excitation of rapid onset. The initial transient burst of activity, when present, was also abolished by the antagonist. Chemodepression evoked by dopamine and chemo-excitation caused by sodium cyanide were unaffected by the doses of MDL 72222 studied, and there were no marked changes in spontaneous discharge or, as judged from preliminary experiments, in the response of the chemoreceptors to alterations in P_{a,O_2}.

Our results show that MDL 72222 is capable of antagonizing the depressant effect of 5-HT on carotid chemosensory discharge and may prove useful in helping to determine the physiological role for 5-HT in the carotid body.

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Effects of the antagonists MDL 72222 and ketanserin on responses of cat carotid body chemoreceptors to 5-hydroxytryptamine

G.C. Kirby & D.S. McQueen

Department of Pharmacology, University of Edinburgh Medical School, Edinburgh EH8 9JZ

1 The effects of intracarotid (i.c.) injections of 5-hydroxytryptamine (5-HT; 1–50 μg) on carotid chemoreceptor activity recorded from the carotid sinus nerve have been studied in anaesthetized cats.

2 Three separate components in the complex response of the chemoreceptors to injected 5-HT were identified. Firstly, a transient burst of activity was obtained during the injection period in 56% of the recordings. Secondly, in all the recordings a period of chemodepression commenced a few seconds after completing the injection and was usually dose-related. Thirdly, a delayed longer-lasting chemoexcitation occurred in many experiments, concomitant with a fall in systemic blood pressure.

3 The neuronal 5-HT receptor antagonist MDL 72222 (10–100 $\mu g\,kg^{-1}$, i.c.) virtually abolished the transient chemoexcitation evoked during 5-HT injections and also significantly increased the mean ID_{50} for 5-HT-induced chemodepression; in 37% of recordings 5-HT caused a dose-related chemoexcitation after the high dose of MDL 72222. Neither the delayed chemoexcitation nor the hypotension caused by 5-HT were much affected by the antagonist. MDL 72222 itself had a biphasic effect on chemosensory discharge, causing depression followed by a delayed excitation.

4 The $5\text{-}HT_2$-receptor antagonist ketanserin (100 $\mu g\,kg^{-1}$, i.c.) had no appreciable effect on the transient chemoexcitation evoked during 5-HT injections and caused a slight but significant increase in the mean ID_{50} for 5-HT-induced chemodepression. The delayed chemoexcitation and accompanying hypotension associated with 5-HT were both substantially reduced or abolished by the antagonist. Ketanserin itself caused a short-lasting period of chemoexcitation.

5 All the effects of injected 5-HT on chemosensory discharge could be abolished by the combination of MDL 72222 and ketanserin (100 $\mu g\,kg^{-1}$, i.c.).

6 Neither MDL 72222 nor ketanserin had any significant effect upon the response of the carotid chemoreceptors to hypoxia. The rate at which discharge increased, and also the steady-state discharge before and during hypoxia, were unaffected by the antagonists, alone or in combination.

7 At least two types of 5-HT receptor appeared to be involved in the response of carotid body chemoreceptors to 5-HT. Transient excitation and chemodepression were mediated via MDL 72222-sensitive (peripheral neuronal) receptors whereas the delayed chemoexcitation and associated hypotension involved a ketanserin-sensitive, presumably $5\text{-}HT_2$-, receptor. It appears unlikely that 5-HT plays a crucial role in chemoreception.

Introduction

Although it is well known that 5-hydroxytryptamine (5-HT, serotonin) is present in the carotid body of many species, including the cat (e.g. Chiocchio *et al.*, 1967), its physiological role there remains to be determined. The effects of exogenous 5-HT on chemosensory activity recorded from the carotid sinus nerve have been studied in anaesthetized cats (Black *et al.*, 1972; Nishi, 1975; Docherty & McQueen, 1978). A complex response is obtained following the intracarotid injection of 5-HT, with a common pattern being a brief period of chemoexcitation followed by a longer-lasting depression of background chemoreceptor discharge. Nishi (1975) found that the response to 5-HT was unaffected by

atropine or hexamethonium, which eliminates involvement of acetylcholine (ACh) receptors, but the putative 5-HT antagonists lysergic acid diethylamide (LSD), gramine and methysergide were also without effect on the response of the carotid chemoreceptors to 5-HT. Categorization of 5-HT receptors in the peripheral nervous system is a complex problem (see Gyermek, 1961; Wallis, 1981) and the value of studies with drugs such as methysergide or LSD in helping to characterize the carotid body 5-HT receptors has been questionable because they lack specificity as 5-HT antagonists. However, the recent advent of more specific 5-HT antagonists such as MDL 72222, shown to be potent in antagonizing the actions of 5-HT at peripheral neuronal sites (e.g. fibres mediating the Bezold-Jarisch reflex in rat – Fozard, 1984), and ketanserin, which is reputed to be a highly selective antagonist at 5-HT_2-receptor sites but inactive at 5-HT_1-receptor sites (Leysen *et al.*, 1981), prompted us to study their effects on the cat carotid chemoreceptors. The aim was to attempt a characterization of 5-HT receptors involved in the response of cat carotid chemoreceptors to 5-HT and, by investigating the influence of the antagonists on responses of the chemoreceptors to a physiological stimulus, hypoxia, obtain information regarding the physiological role of 5-HT in the carotid body.

A preliminary account of some of the results has previously been presented (Kirby & McQueen, 1984).

Methods

Experiments were performed on fifteen cats of either sex, weighing between 2.3 and 3.9 kg, median weight 3.0 kg. Animals were anaesthetized with α-chloralose (65–70 mg kg^{-1}, intravenously) following induction with halothane (5% in oxygen) and supplements of chloralose were administered intravenously as required. In two experiments pentobarbitone (42 mg kg^{-1}, intraperitoneally) was used instead of α-chloralose.

Full details of the experimental procedures have been given previously (McQueen, 1977; Docherty & McQueen, 1978) so only a brief description is provided here. The carotid sinus region on one side was dissected and the ganglioglomerular nerves, which carry the sympathetic nerve supply from the superior cervical ganglion to the carotid body, were cut. The cats were artificially ventilated with room air, apart from periods when hypoxic gas mixtures were used. Gallamine triethiodide (3 mg kg^{-1}) was administered intravenously to prevent spontaneous and drug-induced muscle movements. Drugs were dissolved in modified Locke solution or in 0.9% w/v NaCl solution (saline) and injected, in volumes of 0.1 ml, into the common carotid artery ipsilateral to the sinus nerve from which electrical activity was recorded. They were washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2:95% air at 37°C; injections were generally completed within 1–2 s.

Electrical activity of single or multiple (2–4) chemoreceptor units was recorded from the peripheral cut end of the sinus nerve and stored on tape for subsequent analysis using a pulse height voltage discriminator linked to a microcomputer (McQueen *et al.*, 1984). The units were confirmed as chemoreceptors by their random pattern of discharge, their increase in discharge frequency following injection of sodium cyanide (2.5 μg) into the ipsilateral common carotid artery or in response to hypoxia (breathing 10% oxygen in nitrogen), and by the depression of discharge in response to hyperoxia (breathing 100% oxygen).

Data analysis

A plot of chemosensory discharge (counts per 0.1 s bin) against time was made for each test, and the change in discharge frequency ($\bar{x}$ c.p.s.) from the pre-injection control frequency calculated. In order to standardize results from experiments with different absolute discharge frequencies, the response occurring in the first 5 s of the post-injection period was expressed as a percentage change from control level and plotted against $\log_{10}$ dose. From lines fitted to the dose-response data it was possible to calculate the dose causing a particular response (e.g. ID_{50}, dose causing a 50% reduction in control discharge) and obtain a mean value by pooling data from different experiments.

Hypoxia

The animals were made hypoxic by switching the inspired gas from air to 10% O_2 plus 90% N_2 for 4 min. For each test the control (air-breathing) discharge and steady-state discharge (hypoxia) were measured and arterial blood samples taken before and 3.5 min after onset of the hypoxic stimulus for blood gas analysis. Discharge was measured over consecutive 15 s periods and plotted against time, and a straight line fitted to the values obtained when discharge was increasing in response to the hypoxic stimulus (i.e. until a steady-state maximum (100%) or plateau discharge was obtained). The slope of this line provided an index of the rate of increase in chemoreceptor discharge in response to hypoxia, and was expressed as % max s^{-1}.

Statistics

Mean values are given ± s.e.mean. Statistical analysis of differences between means was carried out using the Wilcoxon two-sample test and the null hypothesis rejected at the 0.05 level of probability (2-tailed).

Drugs

The following compounds were used, and doses are expressed in terms of the salt: 5-hydroxytryptamine creatinine sulphate complex, dopamine hydrochloride (Sigma); MDL 72222 (1αH, 3α, 5αH-tropan-3-yl 3,5-dichlorobenzoate) methanesulphonate salt, kindly donated by Merrell International, Strasbourg; domperidone and ketanserin, tartaric acid salt, kindly donated by Janssen Pharmaceuticals, Beerse, Belgium.

Results

5-HT injections

Sixteen recordings of chemosensory activity were obtained, and intracarotid injection of 5-HT (1–50 μg) consistently caused a *depression* of chemosensory discharge which lasted for 3–15 s. The effect was dose-related in twelve of the recordings (75%), as shown in Figure 1. In the other four experiments chemodepression was not clearly related to dose. 5-HT doses of less than 1 μg had only slight effects on background discharge and these did not differ significantly from those associated with injection of the drug vehicle; the latter had variable effects on chemosensory discharge causing, on average, a 20.5% reduction during the 5 s post-injection period. In nine recordings (56%) chemodepression

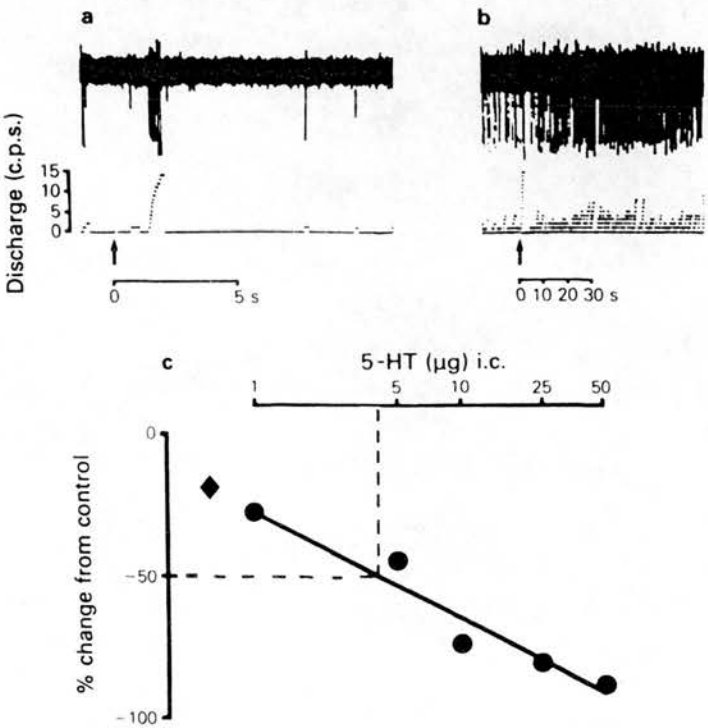

Figure 1 (a and b) Neurograms showing the effects of a single intracarotid (i.c.) injection of 5-hydroxytryptamine (5-HT; 10 μg, at arrow) on chemosensory discharge (3 units) and illustrating in (a) the initial burst of activity which is followed by a period of relative inhibition. A delayed phase of excitation can be seen in (b), which shows the same test displayed at a slower sweep speed. A ramped output below the neurograms gives the number of action potentials counted cumulatively in successive 1 s intervals. (c) Dose-response data showing the relationship between 5-HT dose and the percentage change in chemosensory discharge (from pre-injection control levels) that occurred during the first 5 s after injecting 5-HT. The straight line was fitted to the data by the least squares method, and the ID_{50} (i.e. dose of 5-HT causing a 50% depression of discharge – see broken lines) determined. The mean of the five individual control discharge values, from which the percentage changes were calculated, was 8.03 ± 0.26 c.p.s.. (◆) Effect associated with injection of the drug vehicle.

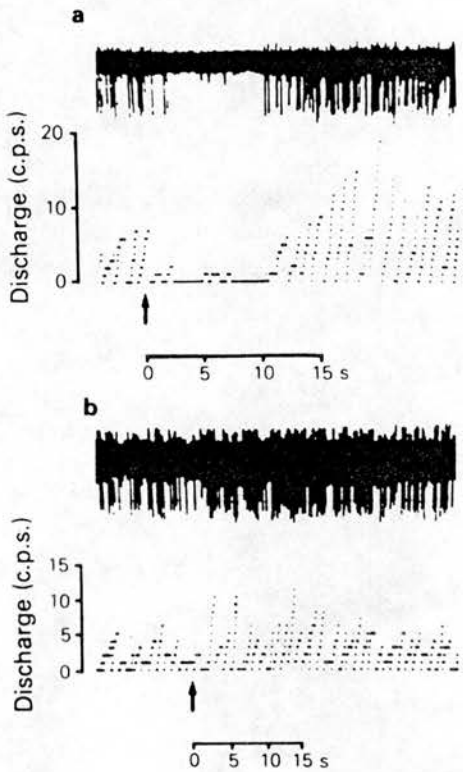

Figure 2 Neurograms of chemosensory discharge illustrating the response to injecting, at the arrow, (a) MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) and, from a separate experiment, (b) ketanserin (100 $\mu g\,kg^{-1}$, i.c.). The ramped trace below each neurogram gives the number of action potentials counted cumulatively in 1 s intervals.

was preceded by a transient burst of chemoreceptor action potentials, usually occurring within the injection period (Figure 1). The threshold for this more variable *excitatory* effect ($\approx 10\,\mu g$) was generally higher than that for chemodepression, but although discharge increased substantially, by 800–1000% in some experiments, a clear dose-response relationship was obtained in only three of the experiments, and the response appeared to be subject to tachyphylaxis. In the remaining 25% of recordings in which 5-HT did not cause transient excitation, chemodepression was still obtained. The averaged ID_{50} for the dose-dependent chemodepression in the recordings where the effect was dose-related was $5.8 \pm 1.9\,\mu g$ ($n = 12$).

In many of the experiments a ***delayed increase*** in chemosensory discharge was observed following the chemodepression (Figure 1). This effect was rather variable, lasted for 10–60 s, and had no consistent dose-response relationship. It appeared to be associated with the fall in systemic blood pressure that occurred following 5-HT injection (Figure 5).

Effects of the antagonist MDL 72222

Intracarotid injection of MDL 72222 (10–100 $\mu g\,kg^{-1}$) caused a depression of background chemosensory discharge which was followed by a delayed increase in discharge frequency (Figure 2a). No chemoexcitation was observed during the period of injection, and systemic blood pressure was not significantly affected by the antagonist. MDL 72222 (10 $\mu g\,kg^{-1}$, i.c.) was studied on seven recordings and in each case the dose-response line relating dose of

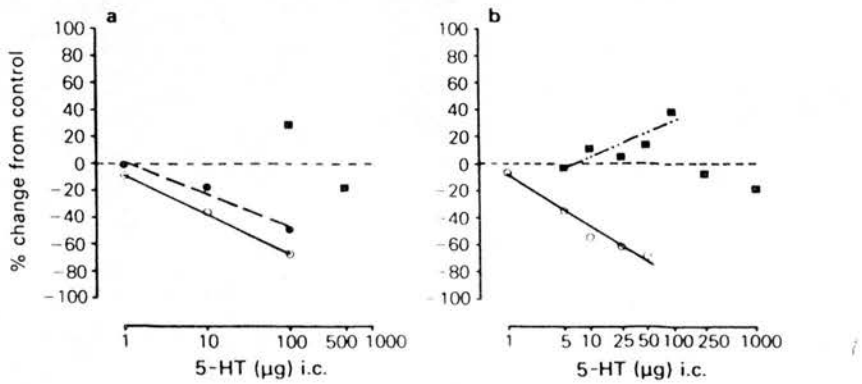

Figure 3 (a) Chemodepressant effect of 5-hydroxytryptamine (5-HT) injected before (○——○) and after (●- - - -●) a low dose of MDL 72222 (10 $\mu g\,kg^{-1}$, i.c.). The rightward shift in the $\log_{10}$ dose-response curve caused by the antagonist is shown. An additional dose of MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) caused a further shift upwards and to the right (■). (b) In a separate experiment the higher dose of MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) completely abolished the 5-HT-induced chemodepression (■···■) and slight chemoexcitation was obtained in response to the lower doses of 5-HT. Lines were fitted to the data by the method of least squares.

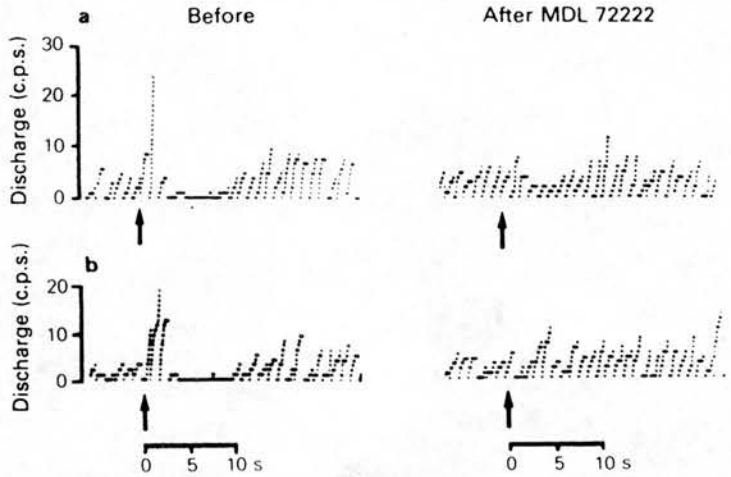

Figure 4 Responses of chemoreceptors (multi-unit recordings) to intracarotid injections of 5-hydroxytryptamine ((a) 5 and (b) 25 μg at arrows) before and after MDL 72222 (100 μg kg^{-1}, i.c.). The initial transient chemoexcitation and the subsequent depression of chemoreceptor discharge were both virtually abolished by the antagonist, and chemoexcitation became evident within the 5 s post-injection period. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

5-HT to chemodepression was shifted upwards and to the right (Figure 3), and the mean ID_{50} was increased to 49.4 ± 33.6 μg ($n = 7$; $P < 0.05$ with respect to controls). When a dose of 100 μg kg^{-1} was given (8 recordings, in 6 of which it followed the lower dose) the ID_{50} increased to 638 ± 408 μg in five (63%) of the recordings. In the other three cases (37%) a dose-dependent chemoexcitation was obtained in the 5 s post-injection period (Figure 3), and no chemodepression occurred unless very high doses of 5-HT (250–1000 μg) were injected. It was possible to calculate an ED_{30} (dose causing 30% increase in discharge above pre-injection level), and this was 103.1 ± 9.0 μg ($n = 3$).

During the nine experiments in which 5-HT caused an initial transient excitation, this part of the response was substantially reduced (totally abolished in two recordings – 22%), by the lower dose of

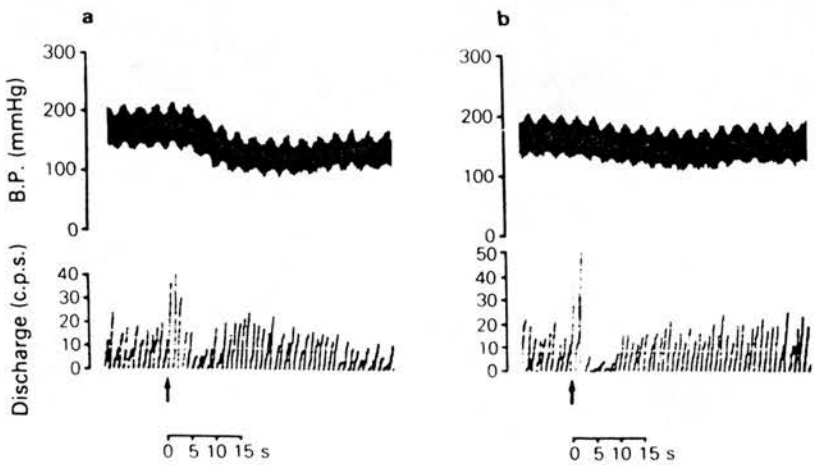

Figure 5 (a) Injection of 5-hydroxytryptamine (5-HT) injected (10 μg i.c., at arrow) affected chemosensory discharge (lower trace, count of action potentials per 1 s interval) and also caused a fall in systemic blood pressure (upper trace). (b) After administering ketanserin (100 μg kg^{-1}, i.c.) the same dose of 5-HT had much less effect upon blood pressure and there was less delayed chemoexcitation 10–15 s after the injection. However, both the initial burst of activity and the subsequent chemodepression were relatively unaffected by the antagonist.

MDL 72222 ($10\,\mu g\,kg^{-1}$), and further reduced or abolished by the higher dose ($100\,\mu g\,kg^{-1}$) – see Figure 4. In one experiment the 5-HT-induced transient chemoexcitation appeared only after MDL 72222 ($10\,\mu g\,kg^{-1}$) and was abolished by adding the higher dose ($100\,\mu g\,kg^{-1}$) of antagonist. The delayed or secondary chemoexcitation, which was more obvious following higher doses of 5-HT, generally increased in magnitude and became more rapid in onset after MDL 72222 ($10-100\,\mu g\,kg^{-1}$), as shown in Figure 6a.

Effects of the antagonist ketanserin

Intracarotid injection of ketanserin ($100\,\mu g\,kg^{-1}$) increased chemosensory discharge in the five recordings studied, an effect which lasted for 10–30 s (Figure 2b), and caused a longer-lasting fall in systemic blood pressure. There was no depression of discharge following ketanserin and no transient chemoexcitation during the injection period. The initial excitation caused by 5-HT was present in three of these recordings and was unaffected by the antagonist (Figure 5). The ID_{50} for 5-HT-induced chemodepression was $14.2 \pm 5.0\,\mu g$ ($n = 4$), which represents a small but significant ($P < 0.05$) decrease in the average response after ketanserin – although much less marked than the antagonism caused by MDL 72222 ($10\,\mu g\,kg^{-1}$), and ketanserin had little or no influence in some of the tests (e.g. Figure 5). In all five recordings the delayed increase in discharge was substantially reduced, as was the hypotensive effect of 5-HT (Figure 5).

Effects of the two antagonists in combination

In five experiments where MDL 72222 ($100\,\mu g\,kg^{-1}$) was administered after ketanserin ($100\,\mu g\,kg^{-1}$) the normal responses to 5-HT injections were absent. Dose-response data for the first 5 s of the responses gave lines of such shallow slope that meaningful ID_{50} or ED_{30} values could only be obtained by extrapolation far beyond the range of doses that could feasibly be used in the experiments, and these were not considered to be meaningful. This was also the case in the five experiments where ketanserin ($100\,\mu g\,kg^{-1}$) was injected after MDL 72222 ($100\,\mu g\,kg^{-1}$).

Responses to dopamine, and the effects of domperidone

The chemodepressant effect of dopamine (0.1–$10\,\mu g$, i.c.) was obtained in all 16 recordings and was unaffected by either ketanserin or MDL 72222 (Figure 6). The dopamine D_2-receptor antagonist domperidone ($10-100\,\mu g\,kg$, i.c.$^{-1}$) was injected in

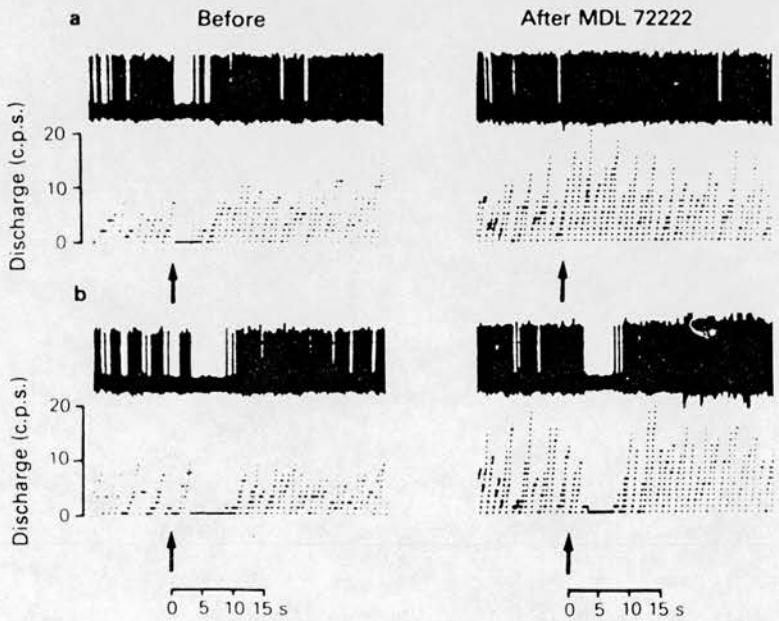

Figure 6 Neurograms showing the response of a single chemoreceptor unit to injections (arrows) of (a) 5-hydroxytryptamine $25\,\mu g$, i.c.) and (b) dopamine ($1\,\mu g$, i.c.) before and after administering MDL 72222 ($100\,\mu g\,kg^{-1}$, i.c.). It can be seen that, whereas the chemodepression evoked by 5-HT was greatly reduced by the antagonist, the dopamine-induced effect was unaltered. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

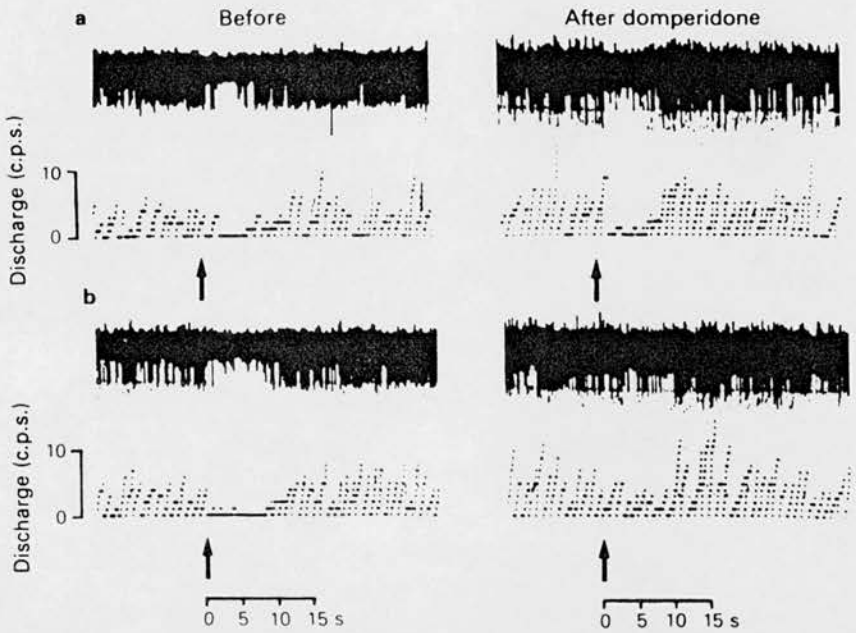

Figure 7 Neurograms showing the responses of chemoreceptor units to injections (arrows) of (a) 5-hydroxytryptamine 10 μg, i.c.) and (b) dopamine (1 μg, i.c.) before and after administering the dopamine antagonist domperidone (10 μg kg^{-1}, i.c.). The antagonist virtually abolished the chemodepressant effect of dopamine but had no appreciable effect upon the response evoked by 5-HT. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

eight preparations and reduced the depressant effect of dopamine on the chemoreceptors without altering the responses to 5-HT (Figure 7), whether injected before (4 experiments) or after (4 experiments) the 5-HT antagonist(s). ID_{50} values for 5-HT-induced chemodepression obtained after domperidone alone were 10.8 ± 2.5 μg ($n = 4$) and 8.1 ± 3.1 μg ($n = 2$) for the 10 and 100 μg kg^{-1} doses, respectively ($P > 0.05$ in comparison with controls).

Responses to physiological (hypoxic) stimulation

The effect of hypoxic stimulation was studied in 14 experiments, and chemoreceptor responses obtained before and after administering MDL 72222 or ketanserin.

MDL 72222 Control chemosensory discharge frequency averaged 6.8 ± 1.5 c.p.s. on air, and increased to 26.8 ± 5.3 c.p.s. on 10% O_2 ($n = 6$). After MDL 72222 (10 μg kg^{-1}) background discharge on air increased to 10.8 ± 3.2 c.p.s., and rose to 27.3 ± 5.8 c.p.s. ($n = 6$) on 10% O_2. The slope of the line relating discharge to time during the period of increasing discharge was 1.08 ± 0.11% max s^{-1} before, and 1.04 ± 0.12 after MDL 72222. The time taken to reach the plateau or steady state discharge (i.e. maximum or 100%) was 112 ± 6 s before, and 109 ± 11 s after the low dose of MDL. A higher dose of MDL 72222 (100 μg kg^{-1}) was studied in 12 experiments (see Figure 8) in three of which ketanserin had previously been injected. Background discharge on air was 5.3 ± 1.0 c.p.s., and it increased to 21.9 ± 3.2 c.p.s. on 10% O_2, the slope being 1.02 ± 0.07% max s^{-1} and the time taken to reach plateau (max) 114 ± 4 s. After MDL 72222 background discharge was 7.7 ± 1.8 c.p.s., discharge on 10% O_2 26.3 ± 5.5, slope 1.08 ± 0.11, and the time taken to reach plateau 116 ± 7 s. None of these parameters was significantly different from control values, following either dose of MDL 72222.

Ketanserin In 6 hypoxia tests performed immediately before the injection of ketanserin (in one of which MDL 72222 had been administered previously) background activity in the pre-ketanserin control state was 6.9 ± 2.0 c.p.s., and increased to 25.6 ± 6.5 c.p.s. on 10% O_2, the slope of the line being 0.99 ± 0.06% max s^{-1}, reaching the plateau or maximum value 112 ± 6 s after changing gases. Following ketanserin (100 μg kg^{-1}) five hypoxia tests were carried out, and there was no significant change

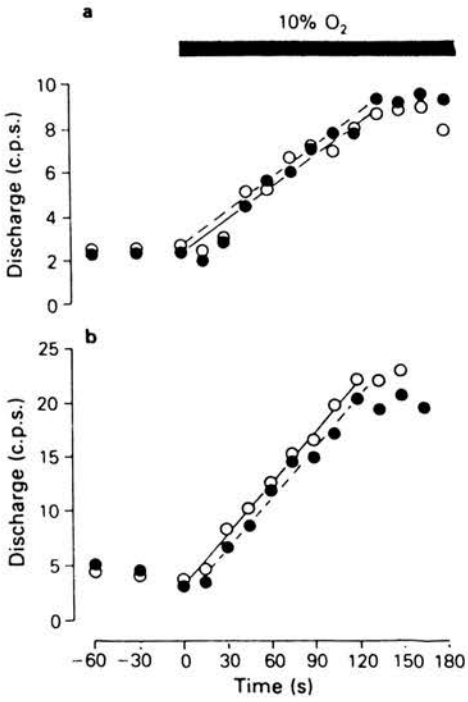

Figure 8 Increase in chemoreceptor discharge caused by ventilating the animals with a hypoxic gas mixture (10% O_2:90% N_2 during the 4 min period starting at 0 s, black bar) instead of room air. (a) Shows the discharge averaged over 15 s intervals, obtained before (O——O) and after (●---●) administering ketanserin (100 $\mu g\,kg^{-1}$, i.c.). (b) Shows the responses obtained, from a separate experiment, before (O——O) and after (●---●) administering MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.). Lines were fitted to the data in the ranges shown by using the least squares method. Neither of the antagonists had any significant effect upon the response to hypoxia.

in any of the parameters (Figure 8). Background discharge was 6.9 ± 2.0 c.p.s., increasing to 26.0 ± 9.5 during 10% O_2, the slope was 1.02 ± 0.14% max s^{-1}, and time to plateau was 116 ± 17 s.

The mean values of arterial blood gas tensions and pH during air breathing (Pa_{O_2} 12.7 ± 0.7 kPa; Pa_{CO_2} 4.3 ± 0.1 kPa; pH 7.30 ± 0.01, $n = 17$) showed no significant differences between control values and those obtained after the administration of antagonists. Similarly, when the animals were made hypoxic, there were no significant differences in blood gas tensions and pH between the values obtained before (Pa_{O_2} 4.8 ± 0.2 kPa; Pa_{CO_2} 4.1 ± 0.2 kPa; pH 7.32 ± 0.01, $n = 17$) and after administration of the antagonists.

Discussion

The present results show that 5-HT has complex and somewhat variable effects upon chemosensory discharge in anaesthetized cats. We identified three separate components in the response to 5-HT and found that the 5-HT antagonists MDL 72222 and ketanserin selectively affected different parts of the response. Previous studies using respiration as an index have provided evidence for both inhibitory and excitatory effects of 5-HT on chemoreceptors in various species including cat (Page, 1952; Douglas & Toh, 1953; Ginzel & Kottegoda, 1954). 5-HT also induces complex neurogenic and circulatory effects which influence respiration independently of the chemoreceptors (Mott & Paintal, 1953; Comroe *et al.*, 1953), which makes the respiratory effect a poor index of chemoreceptor activity. We shall confine ourselves to a consideration of neural data, looking at the effects of antagonists on each of the components of the chemoreceptor response to 5-HT.

Chemoexcitation

Transient chemoexcitation occurred during the injection period in about half the recordings, and this effect has previously been described by Black *et al.* (1972), Nishi (1975), and Docherty & McQueen (1978). The increase was only occasionally dose-related and seemed subject to tachyphylaxis. The rapid onset suggests a direct or perhaps indirect (via release of endogenous substance(s)) action on the sensory nerve fibres (Eyzaguirre & Nishi, 1974), and makes it unlikely to be due to vascular effects of 5-HT. The possible influences on the chemoreceptors of increased sympathetic activity arising from the ganglion-stimulating action of 5-HT (Trendelenburg, 1958) were prevented by sectioning the ganglioglomerular nerves. This does not exclude the possibility that 5-HT may release noradrenaline from the terminals of sympathetic nerves within the carotid body; such an action might be expected to reduce blood flow through the carotid body and could cause a *delayed* increase in discharge. The finding that transient excitation was not obtained in all recordings, in accord with Black *et al.* (1972), could be because fibres differ in their sensitivity: unmyelinated fibres may be more sensitive than myelinated, or the concentration of 5-HT at the receptor site following intracarotid injection may vary between experiments. Tachyphylaxis to 5-HT cannot explain the absence of excitation in response to the initial doses of 5-HT, although in recordings where excitation was occurring, repeated administration of 5-HT in high doses did tend to attenuate the response and may explain why there was no dose-response relationship in many experiments. Nishi

(1975), who evidently found transient chemoexcitation by 5-HT to be more common and induced by lower doses than was our experience, reported that various antagonists were ineffective in blocking the response. He was unable to characterize the receptor responsible for excitation, although he did exclude direct or indirect involvement of nicotinic and muscarinic ACh receptors. Docherty & McQueen (1978) found that the dopamine antagonist α-flupenthixol could reduce excitatory responses to 5-HT, but inconsistently. We have not studied putative 5-HT antagonists such as LSD or methysergide because of concern over their specificity, but instead used the newer 5-HT antagonists MDL 72222 and ketanserin. The latter had no appreciable effect on the transient chemoexcitation but MDL 72222 inhibited it. Thus in some, but not all recordings of chemoreceptor activity, 5-HT increases discharge transiently and this occurs via actions, directly or indirectly mediated, at a receptor, presumably within the carotid body and perhaps associated with sensory nerve endings, which is sensitive to the antagonist MDL 72222. Recent studies have shown that MDL 72222 blocks the excitatory action of 5-HT on the cell bodies of rabbit vagal primary afferents (Azami *et al.*, 1984), and this evidence supports the concept of 5-HT acting on neuronal or sensory receptors in the carotid body. The fact that MDL 72222 itself had some 5-HT-like actions could mean the drug is a partial agonist.

Chemodepression

5-HT caused a short-lasting period of chemodepression which commenced almost immediately upon completion of the injection and was dose-related in the majority of experiments. Again, the rapid onset of the effect makes it unlikely to be secondary to vascular changes caused by 5-HT. It was the most commonly encountered component of the response and occurred regardless of whether or not the initial excitation was present. Depression of chemoreceptor discharge has been demonstrated previously (Black *et al.*, 1972; Nishi, 1975; Docherty & McQueen, 1978). Earlier studies showed that very high doses of α-flupenthixol reduced the relative inhibition caused by 5-HT (Docherty & McQueen, 1978), but none of the putative 5-HT antagonists examined by Nishi (1975) had any effect. His suggestion that depression might be secondary to the initial excitation seems unlikely, for if this were the case, chemodepression should not occur in the absence of an initial depolarization, yet it did in our experiments. We found that ketanserin had a rather variable effect upon 5-HT-induced chemodepression, usually causing a slight reduction (although sometimes a potentiation) of the effect. In contrast, MDL 72222 caused a substantial dose-related antagonism of chemodepression, as shown by the increase in ID_{50} values, and higher doses ($100\,\mu g\,kg^{-1}$) could completely abolish the response, unmasking an excitatory component. Dopamine also causes chemodepression when injected in cats (Docherty & McQueen, 1978), and in view of the fact that high doses of α-flupenthixol can reduce responses to 5-HT as well as to dopamine, we examined the responses to dopamine and 5-HT before and after administering the selective dopamine D_2-receptor (Kebabian & Calne, 1979) antagonist, domperidone. Domperidone had no significant effect on any phase of the chemoreceptor response to 5-HT when given in doses which substantially reduced dopamine-induced chemodepression, and responses to dopamine were unaffected by either MDL 72222 or ketanserin. Thus, we can conclude that chemodepression evoked by 5-HT does not involve a dopamine D_2-receptor and is mainly mediated by mechanisms which are sensitive to MDL 72222. The results with ketanserin could mean that a small part of the depression is attributable to actions on $5\text{-}HT_2$-receptors, assuming the antagonist is selective and does not affect MDL 72222-sensitive sites in the doses studied. Whether the depression of discharge results from direct actions of 5-HT, or is secondary to the release of an inhibitory substance, cannot be determined from our study.

Delayed excitation

The final component of the response to injected 5-HT was a delayed (10–30 s) increase in discharge that lasted longer than any of the other components. However, it was very variable and tended to be concurrent with the fall in blood pressure caused by 5-HT. The antagonist MDL 72222 had no effect on blood pressure responses to 5-HT or on the delayed excitation whereas ketanserin inhibited both the hypotensive effect and the increase in discharge. The chemoexcitation caused by ketanserin alone may reflect some partial agonist activity of the drug. We cannot tell from the data whether the delayed chemoexcitation was due to the hypotension caused by 5-HT, or resulted from actions of the amine on $5\text{-}HT_2$-receptors in the carotid body, perhaps associated with the vasculature (Leysen *et al.*, 1981) or the nerves (e.g. sympathetic terminals – see earlier discussion). Further experiments are needed to resolve the matter.

Classification of 5-HT receptors

In the peripheral nervous system classification of 5-HT receptors is complicated (see Wallis, 1981) and, in addition, new antagonists such as MDL 72222

have yet to be fully characterized *in vivo*. Accordingly, we can only conclude that at least two 5-HT receptors appear to be responsible for changes in carotid chemosensory discharge evoked by injected 5-HT. It is not possible to say whether or not the transient excitation and depression are mediated through a common MDL 72222-sensitive mechanism, but overall our findings are consistent with reports showing that MDL 72222 is a selective antagonist of responses mediated through 5-HT receptors on peripheral nerves (Fozard, 1984) and that ketanserin appears to be an effective antagonist at vascular 5-HT_2-receptors (Leysen *et al.*, 1981).

Physiological stimulation

Although the combination of MDL 72222 and ketanserin antagonized the effects of exogenous 5-HT on chemosensory discharge, the response of the chemoreceptors to physiological stimulation by hypoxia was unaltered. Assuming the antagonists studied reach effective concentrations at sites within the carotid body where locally-released 5-HT acts, the implication is that endogenous 5-HT has no vital role in the mechanism of chemoreception. However, the possibility that 5-HT might exert subtle influences, perhaps as a modulator or co-transmitter, that were not detected in these experiments, cannot entirely be excluded. Neuronal co-storage of 5-HT with polypeptides, some of which are present in the cat carotid body (e.g. substance P; Cuello & McQueen, 1980), and may be neurotransmitters (see Hökfelt *et al.*, 1980), could mean that if 5-HT is involved in chemoreception it may function more as a modulator than as a 'primary' transmitter. The conditions of our experiments do not allow us to reach any conclusions on this possibility, nor on the question of whether 5-HT may be released within the carotid body by efferent nerves. However, the abundance of 5-HT in the carotid body does imply that it has some function in this organ and further studies seem warranted.

Local blood flow within the carotid body may be important in determining chemoreceptor discharge (Joels & Neil, 1963), so 5-HT could be more involved in regulating blood flow than in exerting a direct influence on the chemoreceptor cell-sensory nerve ending complex. Haemodynamic responses to 5-HT may be more subtle than simple changes in vascular tone. For example, 5-HT can increase vascular permeability, resulting in fluid-leakage into the perivascular spaces, and local haemoconcentration ('stasis') within the vessel (Majno & Palade, 1961; Majno *et al.*, 1961). It is doubtful whether such an action would be rapid enough, or sufficiently transient, to explain adequately any of the 5-HT effects we observed, but is indicative of the complex mechanisms that might mediate apparently simple effects of 5-HT. Histochemical studies in rats have confirmed the presence of 5-HT in carotid body type 1 cells, particularly those clustered around blood vessels (Grönblad *et al.*, 1983), so it is conceivable that 5-HT released from type 1 cells acts on 5-HT_2-receptors to alter vascular tone. Such changes in 5-HT output may have important consequences in certain pathophysiological states, such as hypertension (see Steele & Hinterberger, 1972).

In conclusion, 5-HT affects chemosensory discharge and at least two types of receptor appear to be involved in the responses evoked. Further studies with the new specific 5-HT agonists and antagonists, and utilizing neuropharmacological, ligand-binding and histochemical techniques, together with selective denervation of the carotid body, should establish where within this sensory organ these 5-HT receptors are located.

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EFFECTS OF SUBSTANCE P ON RESPONSES OF CAT CAROTID BODY CHEMORECEPTORS TO DOPAMINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE

G.C. Kirby & D.S. McQueen, Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh EH8 9JZ, Scotland, UK

The cat carotid body contains substance P (SP)-like material (Cuello & McQueen, 1980) as well as the putative transmitters dopamine (DA), noradrenaline (NA), and 5-hydroxytryptamine (5-HT). Preliminary investigations showed that SP can modify spontaneous discharge and some drug-induced responses of the carotid chemoreceptors (McQueen, 1980), and we have now studied the influence of SP on responses of cat carotid chemoreceptors to DA, NA and 5-HT. Experiments were performed on pentobarbitone-anaesthetized cats (42 mg kg^{-1} i.p., supplemented as necessary), artificially ventilated and paralysed with gallamine (3 mg kg^{-1} i.v.). Chemosensory discharge was recorded from the peripheral end of a cut sinus nerve, and drugs were injected or infused into the common carotid artery as previously described (McQueen, 1980). Infusions of SP (10 µg min^{-1}) or drug vehicle (Locke solution) were made at a rate of 0.1 ml min^{-1} for 2 min and injections of the amines made 90 s after starting the infusion. This protocol was chosen to minimise the tachyphylaxis caused by SP.

Within 15 s of onset of SP infusion there was a small but significant decrease ($P < 0.05$) in spontaneous discharge from the pre-infusion control frequency; Locke solution had no marked effect on discharge.

NA (0.1 - 50 µg i.c.) caused chemodepression followed by chemoexcitation; chemodepression evoked by the higher, but not the lower doses of NA was potentiated during SP infusion, whereas the delayed chemoexcitation was reduced. 5-HT (1 - 25 µg i.c.) caused dose-related chemodepression that was potentiated during SP infusion. Secondary excitation following chemodepression was smaller in magnitude than that associated with NA, but, in contrast, was potentiated during SP infusion. DA (0.1 - 10 µg i.c.) induced dose-related chemodepression which was greater than that evoked by NA or 5-HT, but the effect was largely unaltered during SP infusion.

Our results indicate that SP-monoamine interactions are rather complex, and there is evidence for a differential effect of the peptide on the responsiveness of carotid chemoreceptors to the amines studied. Thus, SP potentiated NA and 5-HT-induced chemodepression, but had no effect on the depressant action of DA. Delayed chemoexcitation caused by NA was reduced during SP infusion, but that evoked by 5-HT was potentiated. It remains to be established whether SP modifies chemoreceptor activity by acting directly on some element of the chemosensory complex within the carotid body rather than indirectly by a non-specific action (e.g. vascular effects which in turn influence chemoreceptor discharge). Further studies are needed to determine whether the peptide-amine interactions described have any physiological significance, and also to characterize the type(s) of SP receptor involved in modifying chemoreceptor activity.

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SECTION 4

PROSTAGLANDINS, ADENOSINE AND A.T.P.

PAPERS 36 - 46

Prostaglandins and Related Amides 36
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The Modification by Prostaglandin E_1 of Central Nervous Interaction Between Respiratory and Cardio-Inhibitor Pathways

D. S. McQUEEN and A. UNGAR

Department of Pharmacology, The School of Pharmacy, University of of London, Brunswick Square, London

Dogs anaesthetised with morphine, chloralose and urethane often show a marked sinus arrhythmia, the heart accelerating in inspiration and slowing in expiration. This arrhythmia was shown by Anrep et al. (1936) to be partly due to a reflex from the lungs and partly to central irradiation from respiratory to cardio-inhibitor pathways. For our experiments dogs showing marked sinus arrhythmia were selected, and any reflex component of the arrhythmia was excluded by surgical denervation of the lungs in open-chested dogs or by applying positive pressure ventilation at a frequency unrelated to that of the arrhythmia to dogs paralysed by decamethonium.

The cardiac period (duration of the cardiac cycle, beat by beat) was computed electronically from the electrocardiogram. Prostaglandin E_1 (PGE_1) was injected into the common carotid artery in doses of 5-30 nmoles/kg body weight. The arrhythmia was abolished or markedly reduced in every experiment, the cardiac period becoming equal to that in the inspiratory phase of the arrhythmia. PGE thus appears to inhibit an expiratory slowing rather than an inspiratory speeding. The effect, which was not altered by denervation of the carotid sinus region, was apparent within 2.5 seconds of the injection. This establishes a central site of action. The arrhythmia returned after 5-20 minutes.

The central component of the Traube-Hering waves in arterial blood pressure was abolished by PGE_1 in some, but not all experiments. This effect was more transient than the corresponding abolition of sinus arrhythmia.

While the response to PGE_1 was at its height, stimulation of the carotid body chemoreceptors gave rise to a bradycardia similar to one evoked before the injection of PGE_1. The pathways for reflex cardiac inhibition are thus not blocked.

If repeated doses of PGE_1 were injected at 20 minute intervals, tachyphylaxis was seen after two or three injections.

Figure 1 shows the pooled results of our experiments. We have expressed the arrhythmia quantitatively as the standard deviation of the cardiac period divided by the mean cardiac period. This ratio we have called the 'standardised arrhythmia'. The abolition or reduction of the standardised arrhythmia is statistically significant at the probability levels indicated in each of the five animals studied.

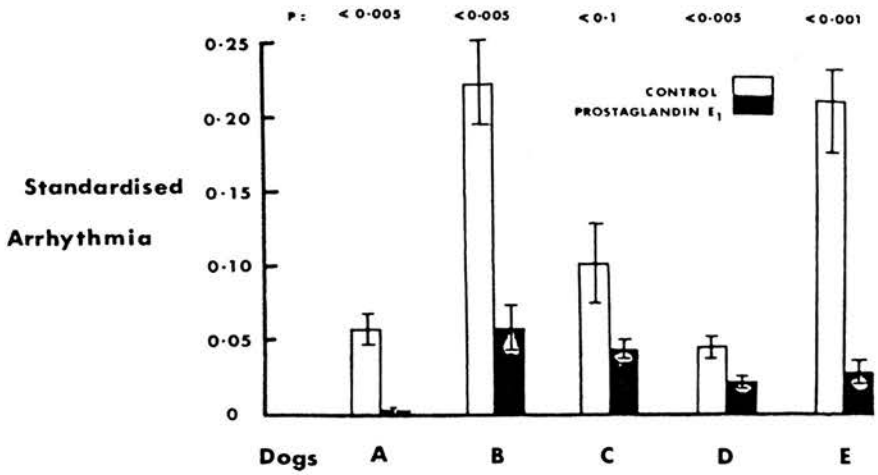

Fig. 1. Inhibition of sinus arrhythmia by PGE_1 Ordinate: standardised arrhythmia = standard deviation of the cardiac period divided by the mean cardiac period. Open columns: control values. Black columns: values after prostaglandin E_1. Abscissa: dogs A to E.

A change in the respiratory pattern was also seen after injection of PGE_1 but this appeared 2 or 3 breaths later than the inhibition of the arrhythmia. There was an increase in pulmonary ventilation, sometimes as great as 100%, due to a reduction in the duration of expiration without any change in other respiratory parameters including duration and amplitude of inspiration and inspiratory and expiratory peak flows. Such responses, isolated to one respiratory parameter are not usually seen with physiological stimuli or respiratory stimulant drugs, but have been described with localised electrical stimulation of the brain stem reticular formation in cats (Hugelin and Cohen, 1963).

Our results suggest that PGE_1 may have a selective action on a group of neurones within the brain stem the effect of which is to inhibit the irradiation of activity from respiratory to cardio-inhibitor and vasomotor pathways.

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The effect of some prostaglandins on respiration in rats and cats

D. S. McQueen (introduced by E. W. Horton)

Department of Pharmacology, University of Edinburgh

It has been noted that certain prostaglandins can stimulate respiration in humans (Bergström, 1967) and dogs (Said, 1967 ; McQueen & Ungar, 1969). This report is on preliminary experiments performed on four cats and ten rats, anaesthetized with pentobarbitone sodium 36 mg/kg, in order to investigate the actions of prostaglandins on respiration.

Prostaglandins were administered by intravenous or intra-arterial infusion and changes in respiratory rate, tidal volume, respiratory minute volume (RMV) and mean arterial blood pressure during and following prostaglandin administration were calculated. Figure 1 shows the response obtained following I.A. administration of prostaglandin E_1 (PGE_1) to a cat.

The increases in RMV began about 15 sec after an infusion of prostaglandin and the effect persisted for up to 5 min in both rats and cats ; PGE_1, PGE_2, PGA_1, all produced increases in RMV. $PGF_{2\alpha}$ and PGA_2 produced variable effects.

Bilateral vagotomy in cats and rats produced very little change in the size of the respiratory response, and in two cats bilateral cutting of the sinus nerves after vagotomy also produced no diminution in the respiratory response to prostaglandin infusion. In one cat, blood pressure compensation (maintaining arterial blood pressure within a few percent of the mean value) did not greatly affect the respiratory response to PGE_1.

Ganglion blocking drugs (hexamethonium bromide 1 mg/kg I.V. ; pentolinium tartrate 0·2 mg/kg I.V.) reduced the respiratory effect following administration of PGE_1 and PGE_2 in rats. In some rats the respiratory response to PGE_2 was reduced before that to PGE_1.

It is concluded that prostaglandins can increase respiratory minute volume in rats and cats, and that the mechanism of action does not appear to involve either the arterial baroreceptors or chemoreceptors. The site of action may be within the central nervous system.

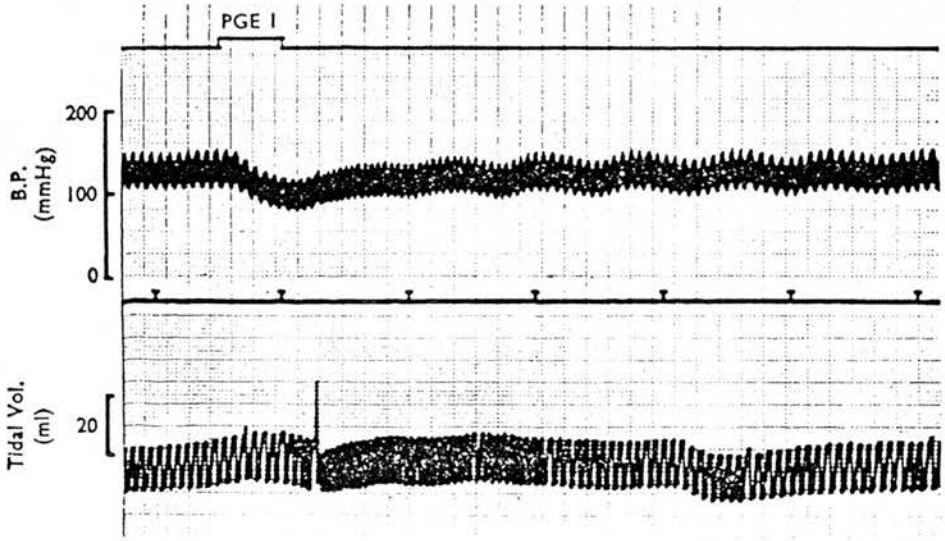

FIG. 1. Cat ♂ 3·6 kg. From above downwards the record shows: marker, I.A. administration of PGE_1, 2·1 μg/kg over a period of 30 sec; arterial blood pressure; one min time marker; and tidal volume.

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THE EFFECTS OF PROSTAGLANDIN E_2, PROSTAGLANDIN $F_2\alpha$, AND POLYPHLORETIN PHOSPHATE ON RESPIRATION AND BLOOD PRESSURE IN ANAESTHETIZED GUINEA-PIGS

Daniel S. McQueen *

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ. Scotland.

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Summary

Prostaglandin E_2 (PGE_2) and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) increased respiratory rate when administered intravenously to anaesthetized guinea-pigs. Polyphloretin phosphate (PPP) at a dose of 40 mg/kg i.v. did not markedly affect either the respiratory response to PGE_2 or $PGF_2\alpha$ nor the vasodepressor effect of PGE_2. The vasopressor effect of $PGF_2\alpha$ was inhibited by PPP. This evidence suggests that the respiratory stimulant property of the prostaglandins is not affected by PPP.

Introduction

Polyphloretin phosphate (PPP), (1), antagonizes the effects of prostaglandin E_2 (PGE_2) and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) on certain smooth muscle preparations (2). It antagonizes the bronchial and blood pressure changes elicited by $PGF_2\alpha$ in the guinea-pig (3) and also protects this species against anaphylactic convulsions (4).

PGE_2 and $PGF_2\alpha$ are both capable of increasing respiratory rate in anaesthetized rats and cats (5) and the present study was undertaken in order to determine whether PPP could influence respiratory changes provoked by PGE_2 or $PGF_2\alpha$ in the guinea-pig.

* Present address: Department of Physiology, University of Utah, Salt Lake City 84112, USA.

Experimental Procedure

Eighteen male guinea-pigs of the Dunkin Hartley strain weighing between 550 and 840 g were used. The animals were fed on diet SG1 (RHM Flour Mills Ltd., Leith, U.K.) with cabbage and water ad lib.

Anaesthetic

Two guinea-pigs were anaesthetized with urethane (ethyl carbamate 25% $^{w}/v$ aqueous solution; 1.2 g/kg i.p.) and the remainder were anaesthetized with pentobarbitone sodium (35 mg/kg i.p.). In most of the experiments it was found necessary to use a local anaesthetic (procaine hydrochloride, 1% $^{w}/v$, 0.5 ml) at the site of the neck incision. A dose of pentobarbitone sufficient to produce surgical anaesthesia generally led to respiratory distress and the animals usually died unless artificially ventilated. If the lungs were artificially ventilated, and the animal survived, it could take as long as three hours after surgery before spontaneous respiration was re-established; results obtained from these animals were not used. There was a very small difference between the dose of pentobarbitone which in conjunction with the local anaesthetic allowed surgery and spontaneous respiration, and that which killed the animals.

Preparation of blood vessels

The animal was placed on a heated table and the trachea was cannulated. The left jugular vein was cannulated with a nylon catheter and this catheter, linked to a three-way tap, was used for intravenous drug administration. The left carotid artery was cannulated with a nylon catheter and the catheter was connected to a blood pressure transducer. Heparin (500 i.u./kg i.v.) was administered to prevent blood clotting.

Recording procedure

The tracheal cannula was connected to a pneumotachograph head linked to an integrating pneumotachograph (Model CS3c, Mercury Electronics Ltd., Newton Mearns, Glasgow, U.K.). A Palmer time clock (2112) reset the pneumotachograph every 10 seconds. A 'staircase' tracing was obtained on a pen recorder which provided a breath by breath record of respiration, the overall height being proportional to the respiratory volume in 10 seconds.

Blood pressure was recorded using a pressure transducer (Consolidated Electrodynamics, L212) connected to the recorder, which was a Devices M4 hot-stylus unit with DC2d pre-amplifiers (Devices Ltd., Welwyn Garden City, Herts., U.K.).

Drug administration

PGE_2 or $PGF_2\alpha$* solutions warmed to 37°C were administered intravenously by infusions of 30 seconds duration every 6-10 minutes using a pump (Model MRHE, Watson Marlow Ltd., Falmouth, Cornwall, U.K.). The stock prostaglandin solutions were 1 mg/ml (in 10% ethanol/90% 0.9% $^{w}/_{v}$ aqueous solution sodium chloride) and these were stored at -20°C. A working solution of PGE_2(10 μg/ml) and $PGF_2\alpha$ (30 μg/ml) was prepared freshly for each experiment by diluting the stock solution with 0.9% $^{w}/_{v}$ aqueous sodium chloride.

One hundred mg PPP** was dissolved in 1 ml distilled water and this solution was either injected over a period of 20 seconds or infused over 5-15 minutes. The solution was re-used after storage at 4°C providing that it was no more than 5 days old.

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* PGE_2 and $PGF_2\alpha$ were kindly supplied by Dr. J. E. Pike, Upjohn Co., Kalamazoo, Michigan.

** Polyphloretin phosphate (PPP; Leo 101 k) kindly supplied by Dr. B. Hogberg, A.B. Leo, Helsingborg, Sweden.

Responses

From the trace obtained, blood pressure and respiratory measurements were made immediately before drug administration (0 seconds, control) and 10, 20, 40, 80, 160 and 320 seconds after the onset of drug administration. Changes were expressed as percentages, the control values being 100%.

Results

Effects of PGE_2 and $PGF_2\alpha$

Both prostaglandins were found to be individually capable of increasing respiratory rate and minute volume on intravenous infusion. A higher dose of $PGF_2\alpha$ was required to produce an increase in respiratory rate comparable to that provoked by PGE_2. PGE_2 caused a marked reduction in blood pressure, while $PGF_2\alpha$ caused a small blood pressure rise. The type of response obtained with PGE_2 can be seen in Fig. 1, A, while that obtained with $PGF_2\alpha$ is shown in Fig. 1, C.

Effects of PPP

PPP (40 mg/kg) was administered intravenously, either as a single dose or as divided doses over 5 minutes or more. Blood pressure fell and respiratory rate increased on PPP administration, this being particularly noticeable on single dose administration, the effect produced being similar to that associated with PGE_2. In some experiments the blood pressure fell so low that the animal died. After PPP had been administered and had exerted its effect, the control blood pressure was slightly reduced and respiratory rate was slightly increased when compared with control values obtained before PPP administration. The blood pressure and respiratory rate changes obtained in response to the injection of single doses of PPP (8 tests, 8 animals) are shown in Table 3.

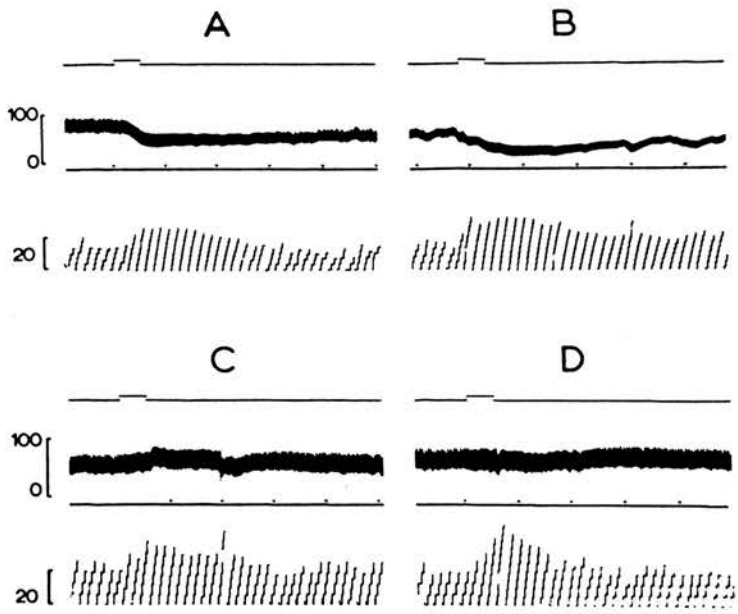

FIG. 1

Legend for Fig. 1: Record, from above downwards:- event marker; blood pressure (0-100 mmHg calibration shown); one minute time marker; breath by breath record of respiration showing respiratory volume every 10 seconds (20 ml calibration shown).

A and B Pentobarbitone anaesthetized guinea-pig, 670 g. PGE_2 12 μg/kg infused at marker before (A) and 15 minutes after (B) PPP 40 mg/kg.

C and D Pentobarbitone anaesthetized guinea-pig 740 g. $PGF_2\alpha$ 58 μg/kg infused at marker before (C) and 15 minutes after (D) PPP 40 mg/kg.

Respiratory rate responses to PGE_2 and $PGF_2\alpha$ were not significantly affected by PPP at 40 mg/kg, it being immaterial whether the prostaglandins were administered 2, 10, 20, 60 or 100 minutes after PPP (see Fig. 1, B; Fig. 1, D). The pooled results for PGE_2 (19 tests in 12 animals before PPP; 17 tests in 8 animals after PPP) and $PGF_2\alpha$ (5 tests in 2 animals before PPP; 5 tests in 2 animals after PPP) are shown in Table 1 (respiratory rate) and Table 2 (blood pressure).

TABLE 1

Respiratory Rate Shown as the Mean Percentages $\pm$ S.E.M. at Various Times After the Onset of Prostaglandin Infusion (Control = 100%). PGE_2 (13 ± 1.3 µg/kg Before PPP 40 mg/kg and 12 ± 0.8 µg/kg After PPP) and $PGF_2\alpha$ (48 ± 4 µg/kg Before PPP 40 mg/kg and 54 ± 1.8 µg/kg After PPP) Were Infused I.V. for 30 Seconds. The Responses Obtained Were Submaximal.

Time(secs)	PGE_2	PGE_2 after PPP	$PGF_2\alpha$	$PGF_2\alpha$ after PPP
0	100	100	100	100
10	107 ± 4.3	112 ± 6.2	169 ± 25	148 ± 17
20	137 ± 6.6	125 ± 7.3	201 ± 42	165 ± 25
40	190 ± 18	178 ± 11	239 ± 30	220 ± 28
80	197 ± 21	182 ± 12	181 ± 17	172 ± 40
160	164 ± 18	138 ± 7.9	157 ± 13	122 ± 14
320	124 ± 9.9	120 ± 5.7	170 ± 29	113 ± 15

TABLE 2

Blood Pressure Shown as the Mean Percentages ± S.E.M. at Various Times After Onset of Prostaglandin Infusion (Control = 100%). Other Details as for Table 1.

Time(secs)	PGE_2	PGE_2 after PPP	$PGF_2\alpha$	$PGF_2\alpha$ after PPP
0	100(56 mmHg)	100(49 mmHg)	100(49 mmHg)	100(45 mmHg)
10	98 ± 1.5	97 ± 1.6	99 ± 1.5	101 ± 2.6
20	89 ± 2.3	92 ± 2.4	102 ± 3.1	103 ± 3.1
40	65 ± 3.7	64 ± 2.8	115 ± 4.7	101 ± 4.5
80	60 ± 3.7	63 ± 3.6	112 ± 12	101 ± 5.4
160	60 ± 4.3	65 ± 4.2	99 ± 8.1	104 ± 6.5
320	68 ± 3.8	79 ± 4.3	88 ± 4.3	106 ± 2.9

Although the vasodepressor response to PGE_2 was not much affected by PPP (the blood pressure recovered more rapidly), the initial rise in pressure with $PGF_2\alpha$ was reduced in magnitude and the later fall (from 160 seconds on) was prevented.

Higher doses of PPP (50-60 mg/kg) in divided doses over 5-15 minutes invariably caused severe respiratory and blood pressure disturbances generally terminating in the death of the animal.

TABLE 3

Respiratory Rate and Mean Blood Pressure Shown as the Mean Percentages ± S.E.M. at Various Times After an Injection of 40 mg/kg PPP (Control = 100%).

Time(secs)	Respiratory rate	Blood Pressure
0	100	100(46 mmHg)
10	114 ± 3.1	88 ± 4.8
20	140 ± 8.7	69 ± 3.9
40	154 ± 13	70 ± 5.0
80	142 ± 9.1	55 ± 4.9
160	100 ± 14	60 ± 7.9
320	95 ± 6.6	90 ± 13

Effect of anaesthetic agent

Two experiments were performed on guinea-pigs anaesthetized with urethane. The respiratory and vascular responses to PGE_2 were similar to those obtained in pentobarbitone anaesthetized animals, both before and after PPP. The respiratory rate of the urethane anaesthetized guinea-pigs was faster (65 breaths/min) than the rate in pentobarbitone anaesthetized animals (35 breaths/min).

Discussion

In the present experiments both PGE_2 and $PGF_2\alpha$ increased respiratory minute volume and respiratory rate following intravenous administration to guinea-pigs anaesthetized either with sodium pentobarbitone or urethane. The respiratory response of the guinea-pig to the prostaglandin infusions is thus similar to that obtained in anaesthetized cats and rats (5) and differs mainly in that $PGF_2\alpha$ is less potent than PGE_2 in evoking the respiratory response.

The blood pressure of guinea-pigs anaesthetized with either pentobarbitone or urethane seemed low (mean 50 mmHg), but this pressure was routinely obtained even in lightly anaesthetized animals, and is in accord with literature values (6). PGE_2 lowered the blood pressure whereas $PGF_2\alpha$ caused a pressure rise, as can be seen in Fig. 1, C.

PPP on single dose administration (40 mg/kg) elicited a respiratory rate increase and a fall in mean blood pressure (See Table 3). In three animals this dose proved fatal, blood pressure falling to zero. PPP given by either single or multiple doses did not markedly affect the respiratory or vascular responses to PGE_2 as can be seen from Tables 1 and 2. The respiratory effect caused by $PGF_2\alpha$ was not much affected (Table 1) but the rise in blood pressure was reduced (Table 2). The ability of PPP to antagonize the pressor effect evoked by $PGF_2\alpha$ in anaesthetized guinea-pigs has previously been reported (3) and confirms that the PPP was active in the present investigation.

The conclusion that PPP does not appear to affect the respiratory rate change provoked by either PGE_2 or $PGF_2\alpha$, nor the blood pressure fall caused by PGE_2, is of interest in that other workers (7) have also found PPP to be incapable of blocking some responses to prostaglandin at dose levels similar to those used in this investigation. Higher doses of PPP were tried, but respiratory distress and very low blood pressure resulted in the death of the animals. It seems that 40 mg/kg of PPP is the highest dose which can be administered to an anaesthetized guinea-pig if the animal is to continue breathing spontaneously.

The results obtained with PPP would seem to suggest that the respiratory stimulating property of the prostaglandins is not secondary to actions at sites where PPP antagonizes prostaglandin-induced changes.

In conclusion, the experimental evidence shows that prostaglandins are capable of stimulating respiration in a variety of species, even when the animals are quite deeply anaesthetized by barbiturates. This effect of the prostaglandins may have a clinical application.

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THE EFFECTS OF SOME PROSTAGLANDINS ON RESPIRATION IN ANAESTHETIZED CATS

D.S. McQUEEN[1]

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

1 Some prostaglandins have been found to be capable of affecting respiration in anaesthetized cats.

2 Prostaglandins E_1, E_2, $F_{2\alpha}$, A_1 and A_2 all elicited increases in respiratory frequency when administered to cats anaesthetized with either pentobarbitone or α-chloralose. This effect was abolished by bilateral vagotomy.

3 Prostaglandins of the E and A series, but not prostaglandin $F_{2\alpha}$, elicited increases in tidal volume which were accompanied by falls in systemic blood pressure in cats anaesthetized with pentobarbitone. The changes in blood pressure were also obtained in cats anaesthetized with α-chloralose, but not the tidal volume changes.

4 It is unlikely that the prostaglandins influenced respiration by direct actions on arterial chemoreceptors or baroreceptors.

5 Mechanisms by which the prostaglandins may be acting to affect respiration are discussed.

Introduction

Prostaglandins are known to be capable of influencing respiration. For example, in anaesthetized dogs prostaglandin E_1 has been observed to increase respiratory rate (Maxwell, 1967; Hirose & Said, 1971) and pulmonary ventilation (McQueen & Ungar, 1969). Experiments in man (Carlson, Ekelund & Orö, 1969) demonstrated that prostaglandin E_1 caused hyperventilation, the increase being mainly due to increased tidal volume, although increased respiratory frequency was observed with higher doses. Other prostaglandins have been found to affect respiration; both prostaglandins E_2 and A_1 increase alveolar ventilation (Said, 1968; Hirose & Said, 1971) while prostaglandin $F_{2\alpha}$ increases respiratory frequency but decreases alveolar ventilation in anaesthetized dogs (Said, Muren & Kirby, 1968). Prostaglandin $F_{2\alpha}$ also increases respiratory frequency in anaesthetized monkeys (White, Heaton & Denton, 1971).

It appears that no detailed investigation of the respiratory changes induced by prostaglandins has been performed and the object of the work described here was to examine some prostaglandins for respiratory effects in anaesthetized cats. The respiratory and vascular responses of anaesthetized cats to various prostaglandins are described and some mechanisms whereby prostaglandins may influence respiration are examined.

A preliminary account has been given of some of the work described in this paper (McQueen, 1972).

Methods

Experiments were performed on 21 cats of either sex weighing between 2.4 and 5.3 kg.

Anaesthesia

Cats were anaesthetized either by an intraperitoneal injection of pentobarbitone sodium (36 mg/kg body weight with supplements of 5 mg/kg body weight given intravenously during the experiment as needed) or by an intraperitoneal injection of α-chloralose, 90 mg/kg body weight. The chloralose was dissolved in hot 0.9% w/v sodium chloride solution (saline) and was cooled to body temperature before injection.

Surgical procedures

In all animals the trachea was cannulated in the neck. Rectal temperature was measured and maintained at 37° C.

A femoral vein was cannulated with a nylon

[1] Present address: Department of Physiology, University of Utah, Salt Lake City, Utah 84132, U.S.A.

catheter, the catheter tip lying in the inferior vena cava close to the junction of the venae cavae. This catheter was used for the intravenous administration of drugs and for the intravenous infusion of prostaglandins.

The right femoral artery was cannulated with a nylon catheter and this catheter was connected to a blood pressure transducer.

The right carotid artery was cannulated at the mid-cervical level with a nylon catheter, the catheter tip being positioned in the ascending aorta about 5 mm rostral to the aortic valves. This catheter was used for the intra-aortic (i. aort.) infusion of prostaglandins. The position of all the catheters was confirmed during post-mortem examination in each animal. In one cat one carotid artery was cannulated both ways and blood drawn from the lower carotid artery was pumped at constant flow to the distal part of the same artery, the contralateral carotid artery having been occluded. Part of the head vascular system was thus perfused at constant flow.

In two cats the left and right femoral arteries were cannulated with short nylon catheters and joined by a Y-piece to a blood pressure compensator. In another cat the abdomen was opened and the abdominal aorta was cannulated between the renal arteries and the inferior mesenteric arteries and connected to the blood pressure compensator. In cats in which a compensator was used, blood pressure was recorded from a brachial artery. The compensator used was as described by McQueen & Ungar (1971).

In three cats, anaesthetized with pentobarbitone and artificially ventilated, the thorax was opened by splitting the sternum in the mid-line. The internal thoracic vessels were ligated. A nylon catheter was positioned in a pulmonary vein via an incision made in the left auricle. This catheter was connected to a blood pressure transducer and a record obtained of pulmonary venous pressure. A similar catheter was positioned in the right ventricle via an incision made in the right auricle. This catheter was connected to a blood pressure transducer and a record obtained of right ventricular pressure. Prostaglandins could be infused into either of these catheters. The chest was closed, the pneumothorax reduced, and the animal allowed to breathe spontaneously. Results from these experiments were not incorporated with those obtained in other experiments because the experimental technique (opening and closing the thorax) differed.

Both vagus nerves were dissected in the neck and loops placed around them preparatory to cutting. In two cats the carotid sinus nerves on both sides were dissected and looped in preparation for sectioning during the experiment.

Recording procedure

In each experiment the tracheal cannula was connected to a pneumotachograph head. In early experiments the pneumotachograph was a Mercury Electronics M3 micromanometer with a V2 continuous integrator, a signal proportional to volume being obtained. In later experiments a Mercury Electronics CS3c integrating pneumotachograph was used in conjunction with a Palmer time clock (2112). A cumulative record of total volume inspired over a period of 30 s was measured and recorded on the pen recorder. A 'staircase' tracing was obtained giving a breath by breath record of respiration, with the overall height being proportional to the respiratory volume in half a minute.

Blood pressure

Arterial or venous blood pressure was recorded by connecting a blood pressure transducer (Consolidated Electrodynamics, Type 4-326-L212) to a catheter and recording the transducer output on a pen recorder (1 mmHg = 1.333 mbar). All animals were injected intravenously with 1000 i.u./kg heparin to prevent blood clotting.

Blood flow

In two cats blood flow in a carotid artery was monitored with an electromagnetic flow probe (MDQ 7020 SCF 2 mm, Statham) connected to a Statham Blood Flow meter (SP 2200). The meter output was recorded by the pen recorder.

Blood gas analysis

In two cats blood from a cannulated femoral artery was sampled before, during and after a prostaglandin infusion. P_aCO_2 P_aO_2 and pH were estimated by means of a Radiometer BMS 3 meter with PHM 71, PHA 930, and PHA 931 attachments.

Recorder

A Devices M4 Hot-stylus recorder with DC2d pre-amplifiers was used. Pens gave a frequency response flat to 75 Hz.

Drugs

The following drugs were used, dissolved in saline unless otherwise indicated:

Prostaglandins E_1 E_2, $F_{2\alpha}$, A_1 and A_2. Stock solution of 1 mg/ml in 10% ethanol: 90% 0.9% w/v aqueous sodium chloride kept frozen (−20° C)

and diluted with saline before use. Working solutions of all the prostaglandins were 10 μg/ml. The prostaglandins used were generously supplied by Dr J.E. Pike of The Upjohn Company, Kalamazoo, Michigan.

Atropine sulphate, histamine acid phosphate, α-chloralose, sodium cyanide (B.D.H. Chemicals Ltd), pentobarbitone sodium (Abbott Laboratories Ltd), mepyramine maleate (May and Baker Ltd), heparin B.P. (Weddel Pharmaceuticals Ltd), propranolol (I.C.I. Ltd) and isoprenaline sulphate B.P. (Boots Ltd).

The doses refer to the salts.

Drug administration

Drugs were either injected, or infused by means of a Watson Marlow MRHE 200 pump. It was noted during the course of the investigation that the response to prostaglandins varied from animal to animal, as did the frequency with which doses of prostaglandin could be administered. In general, a 6 min cycle was followed for prostaglandins E_1 and E_2, with a 10 min time cycle for prostaglandins A_1, A_2 and $F_{2\alpha}$. The doses administered provoked respiratory responses which were submaximal.

Measurement of responses

The responses were measured at 60 s after the start of the 30 s infusion. At this time a maximal respiratory and vascular response was observed, regardless of the dose of prostaglandin administered.

The variables measured were respiratory minute volume (RMV), respiratory rate or frequency (f) and blood pressure (BP). Tidal volume (V_t) was calculated from RMV and f. The results obtained for each set of circumstances (i.e. type of prostaglandin, route of infusion, whether or not vagotomized) were pooled and are expressed as the mean control value ± s.e. mean and the mean incremental change ± s.e. mean.

The dose is expressed as μg/kg, this being the total quantity administered over a period of 30 s at a constant rate of infusion.

Statistical analysis

The null hypothesis that the incremental changes observed were not different from zero was tested using a paired t test on data obtained with prostaglandins E_1 and $F_{2\alpha}$. A t test was applied to incremental changes obtained under different test situations (e.g. i.v.: i. aort; before : after vagotomy). The difference was said to be statistically significant if P was less than 0.05.

Prostaglandins E_2, A_1, and A_2 were found, from a limited number of tests, to evoke changes similar to those seen with E_1. Because the responses to prostaglandin E_1 had been extensively examined, further experiments with these other prostaglandins were not performed.

Results

Prostaglandins E_1 and $F_{2\alpha}$ were investigated for their ability to evoke respiratory changes. Prostaglandins E_2, A_1 and A_2 were found to be similar to E_1 in their ability to affect respiration, and the results obtained with these prostaglandins will be described qualitatively. Prostaglandins A_1 and A_2 were about twice as potent as E_1 in eliciting respiratory rate increases and hypotension. The main difference between the E and A series was that the latter caused sizeable falls in BP when infused i.v., comparable with the effect seen on i. aort. infusion of the same dose.

Prostaglandin E_1

Data obtained from experiments with prostaglandin E_1 are summarized in Table 1. When infused either i.v. or i. aort. prostaglandin E_1 evoked a dose-dependent increase in f and RMV; V_t increased on i. aort. infusion but decreased on i.v. administration. The change in f started about 10 s after the start of an i.v. infusion and continued for the duration of the infusion, at least up to 4 min which was the longest infusion period studied. The rate increase was sometimes preceded by one deep breath or by a period of respiratory inhibition (Figure 1).

On i. aort. infusion prostaglandin E_1 evoked an increase in f, an increase in V_t and a fall in BP. The f increase started about 24 s after the start of the infusion and the V_t increase was greatest at the time when the hypotension was maximal (Figure 2). The f increase obtained on i.v. administration of prostaglandin E_1 was significantly bigger than that obtained on i. aort. administration ($P < 0.05$) although smaller doses of prostaglandin E_1 were administered i. aort. before vagotomy in order to avoid obtaining large vascular effects. In those experiments in which equal doses were infused by the two routes, the f response was always greatest on i.v. infusion. The RMV increase was greatest on i. aort. infusion ($P < 0.01$) and the BP fall was greatest with i. aort. infusions ($P < 0.01$).

Bilateral vagotomy significantly reduced the f increase ($P < 0.01$ for either route) but the V_t increase observed on i. aort. infusions was still

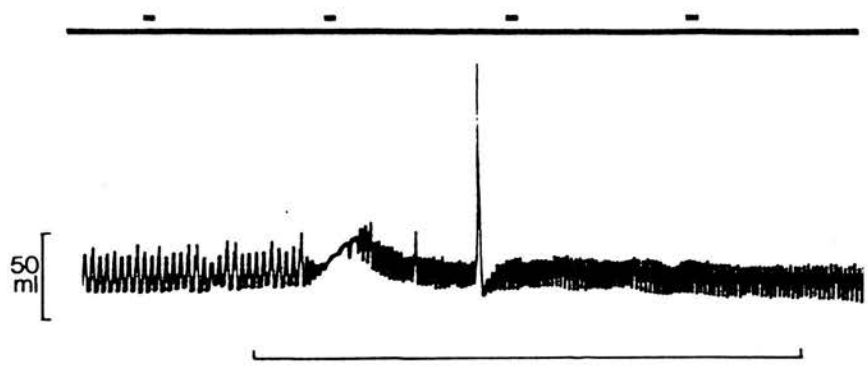

Fig. 1 Cat (male, 4.4 kg) anaesthetized with pentobarbitone. Prostaglandin E_1, 12 μg/kg, infused over a period of 3 minutes. Record, from above downwards: 1 min time marker; breath by breath record of respiration; marker indicating period during which prostaglandin E_1 was infused.

present after vagotomy, although reduced in magnitude. The i. aort. RMV increase was significantly reduced by vagotomy ($P < 0.01$) and bilateral vagotomy also resulted in prostaglandin E_1 evoking a bigger depressor response ($P < 0.05$ pre/post vagotomy) although the response to i. aort. infusions was not significantly affected.

In two experiments measurements of P_aCO_2 were made before and during the RMV increases evoked by prostaglandin E_1 infusions and it was found that alveolar hyperventilation was occurring since the P_aCO_2 fell.

Prostaglandin $F_{2\alpha}$

Data obtained from experiments with prostaglandin $F_{2\alpha}$ are summarized in Table 2. Prostaglandin $F_{2\alpha}$ when infused i.v. increased respiratory rate, this increase often being prolonged following a 30 s infusion (Figure 3). No significant change in

Table 1 Prostaglandin E_1 (PGE_1) infused i.v. or i. aort. before and after bilateral vagotomy, pentobarbitone anaesthesia

	Before vagotomy			*After vagotomy*		
	Control	*Increment*	P	*Control*	*Increment*	P
			Intravenous			
f (breaths/min)	22 ± 2.1	9.5 ± 1.0	<0.01	17 ± 1.3	0.4 ± 0.5	•
V_t (ml)	30 ± 1.7	−1.6 ± 0.6	<0.01	38 ± 3.1	4.2 ± 1.7	<0.05
RMV (ml/min)	660 ± 36	160 ± 20	<0.01	570 ± 44	104 ± 41	<0.05
BP (mmHg)	140 ± 2.7	−17 ± 1.8	<0.01	130 ± 7.1	−24 ± 3.8	<0.01
			Intra-aortic			
f (breaths/min)	16 ± 2.1	5.7 ± 0.9	<0.01	16 ± 0.4	1.7 ± 0.9	•
V_t (ml)	33 ± 1.4	22 ± 6.8	<0.01	37 ± 5.3	7.8 ± 2.5	<0.05
RMV (ml/min)	520 ± 72	610 ± 120	<0.01	590 ± 93	180 ± 54	<0.05
BP (mmHg)	140 ± 6.8	−53 ± 4.1	<0.01	130 ± 14	−52 ± 9.5	<0.01

i.v. before vagotomy 59 tests in 10 cats. Dose PGE_1 = 3.4 ± 0.2 μg/kg
i.v. after vagotomy 21 ,, ,, 8 ,, ,, ,, 3.9 ± 0.4 ,,
i. aort. before vagotomy 12 ,, ,, 4 ,, ,, ,, 1.9 ± 0.2 ,,
i. aort. after vagotomy 8 ,, ,, 5 ,, ,, ,, 2.8 ± 0.3 ,,
Data are shown as mean ± s.e. • indicates $P > 0.05$.

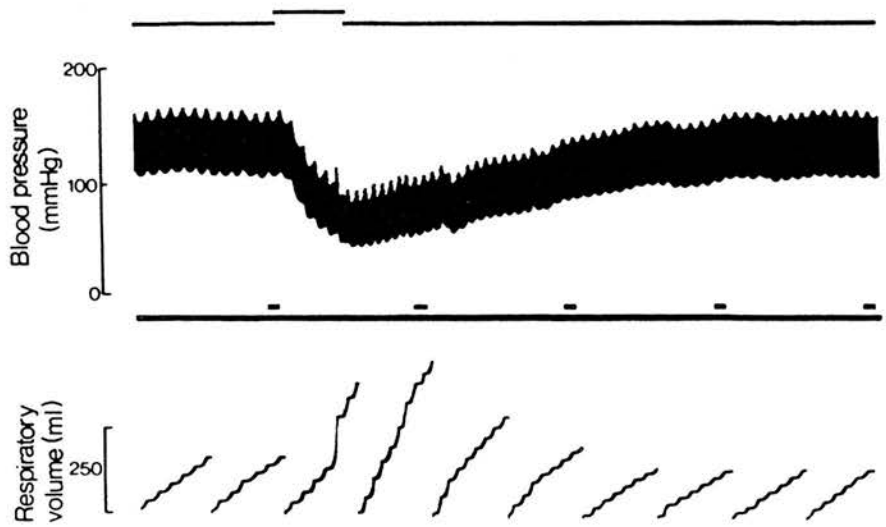

Fig. 2 Cat (male, 3.5 kg) anaesthetized with pentobarbitone. Prostaglandin E_1, 0.9 μg/kg, infused i. aort. over a period of 30 seconds. Record, from above downwards: event marker; arterial blood pressure; 1 min time marker; breath by breath record of respiration, total height representing the respiratory volume in 30 seconds.

f was observed on i. aort. infusion either before or after vagotomy and there was no significant change in V_t following prostaglandin $F_{2\alpha}$ administration by either route, and vagotomy did not alter this. Blood pressure fell slightly when prostaglandin $F_{2\alpha}$ was infused i.v. but not when infused i. aort. The f increase seen on i.v. administration was markedly reduced after bilateral vagotomy ($P < 0.01$).

Chloralose anaesthesia

A series of experiments was performed on cats anaesthetized with α-chloralose in order to

Table 2 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) infused i.v. or i. aort. before and after bilateral vagotomy, pentobarbitone anaesthesia

	Before vagotomy			*After vagotomy*		
	Control	*Increment*	P	*Control*	*Increment*	P
			Intravenous			
f (breaths/min)	19 ± 1.4	6.4 ± 1.1	0.01	14 ± 1.0	0.7 ± 0.8	•
V_t (ml)	32 ± 2.5	−1.7 ± 1.4	•	49 ± 2.2	3.5 ± 3.9	•
RMV (ml/min)	540 ± 32	160 ± 49	0.01	660 ± 45	79 ± 84	•
BP (mmHg)	130 ± 4.3	−10 ± 4.8	0.05	130 ± 10	−3.1 ± 6.1	•
			Intra-aortic			
f (breaths/min)	17 ± 1.8	3.7 ± 1.7	•	16 ± 0.9	−1.5 ± 1.3	•
V_t (ml)	33 ± 0.7	5.0 ± 2.1	•	44 ± 3.6	2.5 ± 5.9	•
RMV (ml/min)	540 ± 55	220 ± 55	0.05	700 ± 63	−22 ± 110	•
BP (mmHg)	130 ± 10	−2.2 ± 5.3	•	130 ± 11	−8.1 ± 8.7	•

i.v. before vagotomy	23 tests in 8 cats.	Dose $PGF_{2\alpha}$ =	3.2 ± 0.3 μg/kg
i.v. after vagotomy	6 ,, ,, 3 ,,	,, ,,	3.0 ± 0.4 ,,
i. aort. before vagotomy	7 ,, ,, 4 ,,	,, ,,	2.6 ± 0.4 ,,
i. aort. after vagotomy	8 ,, ,, 5 ,,	,, ,,	3.2 ± 0.5 ,,

Data are shown as mean ± s.e. • indicates $P > 0.05$.

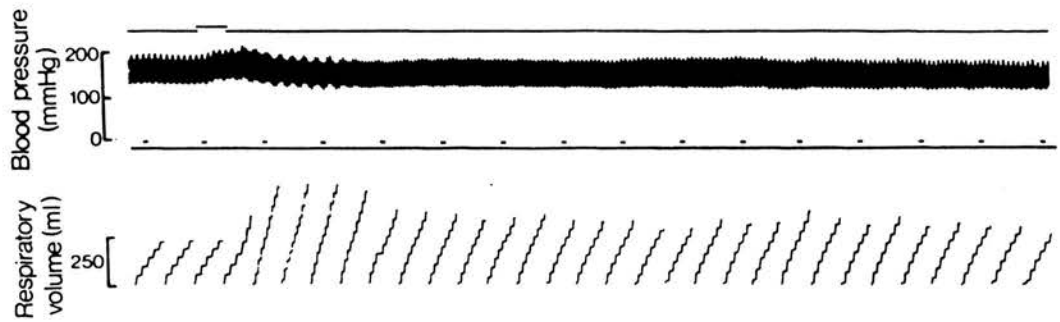

Fig. 3 Cat (female, 2.9 kg) anaesthetized with pentobarbitone. Prolonged response to an infusion of prostaglandin $F_{2\alpha}$ (4.1 μg/kg over a 30 s period) into the abdominal aorta. Record, from above downwards: event marker; arterial blood pressure; 1 min time marker; breath by breath record of respiration, total height representing the respiratory volume in 30 seconds.

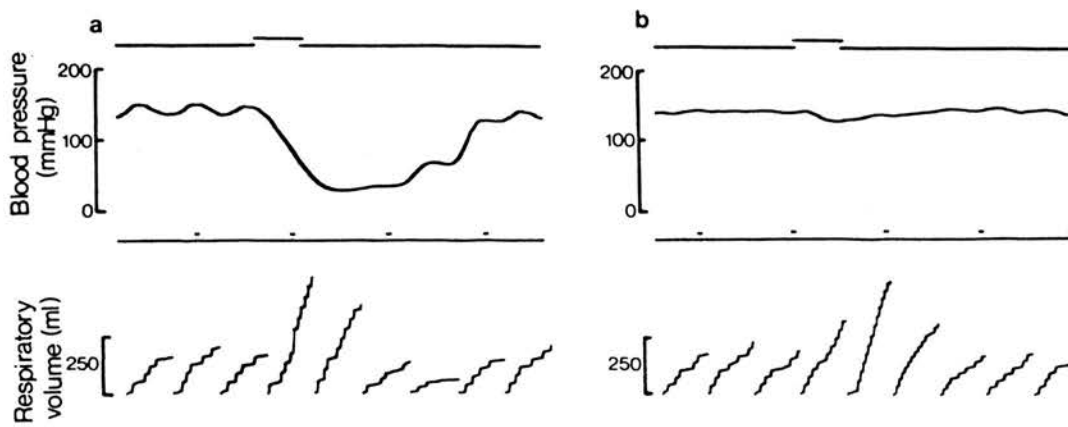

Fig. 4 Cat (male 3.0 kg) anaesthetized with pentobarbitone. (a) Prostaglandin E_2, 2.7 μg/kg, infused i. aort. over a 30 s period; (b) prostaglandin E_2, 2.7 μg/kg, infused i. aort. over a 30 s period, with blood pressure compensator connected to the abdominal aorta. Record details as in Fig. 2, excepting that the mean blood pressure is shown.

examine the possibility that the changes observed in cats anaesthetized with pentobarbitone were peculiar to the anaesthetic agent. Some of the results obtained are summarized in Table 3. Prostaglandin E_1 evoked an increase in respiratory rate when administered either i.v. or i. aort. as did $F_{2\alpha}$ i.v., and these increases were significantly reduced after vagotomy ($P < 0.01$). V_t fell on i.v. administration, but there was no significant change when the prostaglandin was infused i. aort. RMV was not significantly affected by prostaglandin E_1 i.v., but it was increased by E_1 or $F_{2\alpha}$ administered i. aort. BP fell when prostaglandin E_1 was infused i.v. and a bigger fall was obtained when it was infused i. aort. ($P < 0.01$). Prostaglandin $F_{2\alpha}$ did not significantly affect blood pressure.

Blood pressure compensation

In three cats anaesthetized with pentobarbitone the mean arterial blood pressure was compensated. The results obtained are illustrated by Figure 4. BP compensation reduced the increase in V_t and a larger increase in f was obtained. After bilateral vagotomy BP compensation reduced the tidal volume increment evoked by prostaglandin E_1 by about 60%.

Blood flow to the head

When carotid blood was pumped to the head at constant flow changes in respiration followed the intravenous or intra-arterial (descending aorta)

Table 3 Cats anaesthetized with chloralose. Infusions of prostaglandin E_1 (PGE_1) or $F_{1\alpha}$ ($PGF_{1\alpha}$)

	PGE_1 i.v.			PGE_1 .. aort.			$PGF_{1\alpha}$ i.v.		
	Control	*Increment*	P	*Control*	*Increment*	P	*Control*	*Increment*	P
f (breaths/min)	15 ± 0.7	5.1 ± 1.2	<0.01	15 ± 0.7	3.5 ± 0.8	<0.01	16 ± 0.5	7.1 ± 1.6	<0.01
V_t (ml)	26 ± 1.5	−3.5 ± 0.8	<0.01	21 ± 1.2	4.0 ± 2.1	*	27 ± 2.2	−4.5 ± 1.4	<0.01
RMV (ml/min)	380 ± 27	65 ± 35	*	310 ± 23	160 ± 49	<0.05	440 ± 33	79 ± 24	<0.01
BP (mmHg)	130 ± 5.8	−23 ± 4.9	<0.01	130 ± 3.3	−67 ± 3.1	<0.01	120 ± 3.8	−7.8 ± 10	*

PGE_1 i.v. 16 tests in 5 cats. Dose = 3.7 ± 0.5 μg/kg
PGE_1 i. aort. 7 " " 3 " " 3.5 ± 0.6 "
$PGF_{1\alpha}$ i.v. 13 " " 4 " " 3.6 ± 0.4 "
Values are shown as the mean ± s.e. $P > 0.05$ = *.

Fig. 5 Vagotomized cat (female, 3.6 kg) anaesthetized with pentobarbitone, showing the response to prostaglandin E_1 (3.1 μg/kg infused over a 30 s period) obtained before (stippled column) and after (solid column) bilateral sinus nerve section.

administration of prostaglandin E_1; injection of prostaglandin E_1 into the head perfusion circuit did not evoke any respiratory changes until the prostaglandin had had time to recirculate. It appears, therefore, that prostaglandins do not affect respiration by actions within the vascular territory perfused by the carotid arteries, and also that even when part of the CNS is perfused at constant flow, respiratory changes are still obtained.

Experiments with an electromagnetic flow probe positioned around a common carotid artery revealed that flow in this vessel increased on i.v. administration of prostaglandin E_1 but decreased on i. aort. administration. Flow in the vertebral arteries would be expected to follow the direction of flow in the carotid arteries, and so it may be concluded that blood flow to the CNS increases during i.v. prostaglandin E_1 administration, even though systemic blood pressure falls, and that blood flow to the CNS falls during i. aort. administration.

Carotid sinus denervation

In two experiments on vagotomized cats anaesthetized with pentobarbitone the respiratory response to prostaglandin E_1 was examined before and after bilateral sectioning of the carotid sinus nerves. The results obtained are illustrated by Figure 5. The RMV increment was further increased following sinus denervation and this was associated with a larger fall in blood pressure and an increase in V_t. The evidence argues against any direct activation of either the carotid chemoreceptors or baroreceptors.

Pulmonary vascular responses

In three cats anaesthetized with pentobarbitone no significant change in central venous pressure was observed for any of the prostaglandins administered by either the venous or arterial route.

Catheters were positioned in a pulmonary vein and in the right ventricle in three cats. Pressure recordings in the spontaneously breathing animals showed no consistent change in right ventricular pressure with prostaglandins E or A, although $F_{2\alpha}$ caused a rise in pressure. Pulmonary venous pressure was reduced slightly by prostaglandin $F_{2\alpha}$, increased slightly by E_1 and E_2 and not affected by A_1 or A_2 on i.v. infusion.

Infusions of prostaglandin into the right ventricle evoked respiratory changes similar to those seen on i.v. administration, and infusion into a pulmonary vein elicited a response akin to that seen on i. aort. administration. The 'receptors' for the *f* response seem therefore to be located in the vascular territory between the right ventricle and the larger pulmonary veins.

Blocking drugs

Mepyramine. It has been suggested (Willis, 1969) that prostaglandin E_1 may act in some situations by releasing histamine from mast cells. In the present work two experiments were performed on pentobarbitone anaesthetized cats in which prostaglandin E_1 was infused before and after 0.33 mg/kg mepyramine, a dose which blocked the vascular response to injections of histamine. The respiratory effect evoked by prostaglandin E_1 was not markedly altered by mepyramine, thereby suggesting that this prostaglandin does not produce its respiratory effect via histamine release. It is also unlikely that it acts by liberating 5-hydroxytryptamine (Thompson & Angulo, 1969).

Propranolol. In two vagotomized cats anaesthetized with pentobarbitone, isoprenaline elicited an increase in RMV, due mainly to increased V_t, and a BP fall. Prostaglandin E_1 produced similar effects, except that the RMV change was associated with an *f* increase. Propranolol (0.25 mg/kg i.v.) abolished the vascular and respiratory responses to isoprenaline without altering the changes evoked by prostaglandin E_1. It is unlikely, therefore, that prostaglandin E_1 produces its effects by actions, direct or indirect, at β-adrenoceptors.

Discussion

Prostaglandins have been shown in the present study to be capable of affecting respiration in anaesthetized cats. The findings are in general agreement with observations made in other species (see Introduction).

The results obtained make it possible to eliminate certain potential mechanisms from involvement in the respiratory response to prostaglandin administration. Thus, the respiratory stimulating property of the prostaglandins examined did not appear to be associated with actions at histamine receptors or β-receptors; indirect evidence suggests that 5-hydroxytryptamine is unlikely to be involved. Direct actions of the prostaglandins on either the carotid baroreceptors or chemoreceptors also appeared unlikely, and McQueen & Belmonte (1974) have since confirmed this using electrophysiological techniques. The possibility that the respiratory changes observed were secondary to changes in bronchial structures was considered. It is unlikely that the *f* change, the effect most associated with the pulmonary system in the present work, is secondary to changes in bronchial tone because Hirose & Said (1971) demonstrated that in dogs anaesthetized with pentobarbitone, prostaglandin E_1 evokes an increase in *f* without altering dynamic lung compliance or airway resistance. However, it is possible that the cat may differ from the dog in this respect.

It has not been possible in the present investigation to determine how the prostaglandins elicit the respiratory changes observed, but some evidence has been obtained which implicates certain mechanisms and this will be discussed below in terms of V_t and *f* changes.

Tidal volume

The results demonstrated that the tidal volume increase was usually preceded by a fall in mean blood pressure (see Figure 2). Prostaglandin $F_{2\alpha}$ which did not possess much depressor activity, did not cause a significant increase in V_t. Lowering the BP by use of a compensator or a dilator drug such as isoprenaline also increased V_t. The fall in BP and the increase in V_t were both greatest when the prostaglandin (E or A series) was administered into the ascending aorta. Results from experiments with a blood pressure compensator (Fig. 4) suggest that the BP fall evokes an increase in V_t, this increase tending to mask part of the rate increase elicited by the prostaglandin. It is known that a reduction in blood flow to the CNS in cats stimulates respiration, while an increase in flow depresses respiration (Schmidt, 1928). A possibility is therefore that prostaglandins which increase V_t do so at least partly as a consequence of reduced blood flow to the CNS, and this is supported by the evidence that the V_t increase is associated with reduced blood flow in the carotid

artery. However, blood pressure reduction also removes inhibitory tone from the baroreceptors and such removal leads to an increase in respiration (Heymans & Neil, 1958). It will be necessary to perform further studies in order to determine the extent to which flow changes, removal of inhibitory baroreceptor tone, or other mechanisms contribute to the V_t response.

It appears that the V_t changes are not seen in animals anaesthetized with α-chloralose, although the blood pressure fall is observed on prostaglandin administration. The reason for this difference between α-chloralose and pentobarbitone will need further investigation.

Respiratory frequency

The f increase was observed with all the prostaglandins examined, under both pentobarbitone and α-chloralose anaesthesia. It was most apparent on i.v. administration and took about 14 s longer to develop on i. aort. administration. The region from which the response appeared to be most readily elicited lay between the right ventricle and the large pulmonary veins. These observations, coupled with the finding that the f response was virtually abolished after bilateral vagotomy, suggests that the effect may be due to actions at a sensory receptor with vagal afferents.

In the present study it has not proved possible to establish the precise site and type of receptor which may be involved in the f response. Daly, Ludnay, Todd & Verney (1937) and Aviado, Li, Kalow, Peskin, Turnbull & Hess (1951) described receptors in the pulmonary venous system of dogs which provoked an increase in respiratory rate when activated by increased pulmonary venous pressure. However, only slight and variable effects on pulmonary vascular pressure were observed in the present experiments. The possibility that extensive changes in tone of pulmonary venules might occur without this being reflected in changes of pulmonary venous pressure should be borne in mind.

The receptors described by Paintal (1955) are unlikely to be involved because the delay in onset of the f response after i.v. administration of prostaglandin, 10 s, is longer than would be anticipated for direct stimulation of these receptors, unless a prostaglandin metabolite is the active agent.

It might be argued that bilateral vagotomy so alters respiratory control that it becomes difficult to establish whether prostaglandins are acting via vagal afferents to affect f. Electrophysiological experiments could be performed in order to investigate directly the question of whether prostaglandins affect pulmonary sensory receptors.

The possibility of prostaglandin actions within the brain stem has not been excluded by the present study and should also be investigated.

Respiratory changes can cause reflex alterations through the lung inflation reflex (Scott, 1966). When examining prostaglandins for effects in whole animals it would seem advisable to note that prostaglandins are capable of eliciting respiratory changes.

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THE EFFECTS OF PROSTAGLANDINS E_2, A_2 AND $F_{2\alpha}$ ON CAROTID BARORECEPTORS AND CHEMORECEPTORS.

By D. S. McQUEEN*, and C. BELMONTE†. From the Department of Physiology, University of Utah College of Medicine, Salt Lake City, Utah 84132.

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The actions of prostaglandins E_2, A_2 and $F_{2\alpha}$ on the sensory receptors of the carotid bifurcation have been investigated. Electrical activity of baroreceptor and chemoreceptor fibres was recorded from the sinus nerve of anaesthetized cats. Prostaglandins elicited alterations in blood pressure which were accompanied by changes in baroreceptor activity. Chemoreceptor spontaneous activity was modified by the injection of prostaglandins; such variation did not occur *in vitro* and thus appears to be secondary to changes in carotid blood flow. We have been unable to demonstrate any action of the prostaglandins at the concentrations studied on baroreceptors or chemoreceptors of the carotid bifurcation, apart from changes secondary to vascular effects.

There are reports based on indirect evidence which suggest that prostaglandins can affect sensory receptors in the carotid artery. For example, Carlson and Orö [1966] have proposed that prostaglandin E_1 (PGE_1) may have 'direct actions on the carotid artery or on structures in this artery' to evoke reflex vascular changes in dogs. Kaplan, Grega, Sherman and Buckley [1969] have reported that in dogs part of the hypotensive response to PGE_1 administration results from actions on the carotid sinus-body structures, most likely the baroreceptors. However, McQueen [1972] examined the respiratory and vascular responses of the cat to various prostaglandins and concluded that it is unlikely that either the carotid baroreceptors or chemoreceptors are directly involved in the responses, but without electrophysiological evidence could not preclude the possibility.

The object of this study was to establish directly whether prostaglandins affect sensory discharges recorded from baroreceptor and chemoreceptor units in the carotid sinus nerve of the cat. The results indicate that the observed changes in baroreceptor and chemoreceptor activity are secondary to vascular changes, and that prostaglandins in the concentrations studied do not affect directly the sensory receptors of the carotid bifurcation.

METHODS

Cats of either sex (2·0–2·7 kg) were anaesthetized with sodium pentobarbital (Diabutal, 40 mg/kg i.p.). A femoral vein was cannulated and used for drug administration, the catheter tip lying in the mid-abdominal region. A cannula was inserted into the

* Dorothy Temple Cross Research Fellow from Department of Pharmacology, University of Edinburgh, Scotland.

† N.I.H., International Post-Doctoral Fellow. Present address: Depto. de Fisiología, Facultad de Medicina, Valladolid, Spain.

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trachea low in the neck and connected to a demand valve respirator (Ensco). Gallamine triethiodide (Flaxedil, 1 mg/kg) was administered i.v. to paralyse spontaneous respiration. In some experiments a comparison of chemoreceptor activity, both spontaneous and drug-induced, was made before and after gallamine administration. The chemoreceptor discharges before gallamine were similar to those obtained after administration of the drug and this is in agreement with other reports [see Torrance, 1968]. The animal was ventilated with air and the end-tidal CO_2 was continuously monitored by an infra-red CO_2 analyzer (Beckman, LB-1), the P_{CO_2} being maintained at 20–25 mm Hg (atmospheric pressure = 638–650 mm Hg) by adjusting the frequency and volume of respiration.

The carotid nerve and carotid body were dissected from surrounding tissues under a binocular microscope. The ganglioglomerular nerves were cut and the carotid nerve was sectioned centrally and placed on bipolar platinum-iridium electrodes for recording. Fine strands were dissected from the nerve trunk when recordings of single baroreceptor or chemoreceptor units were required. Exposed tissues were covered with warm mineral oil. The lingual artery on the same side as the sinus nerve from which the recordings were to be made was cannulated, the tip of the catheter lying about 2 cm below the carotid bifurcation.

Sensory nerve discharges were amplified, displayed on an oscilloscope and recorded on magnetic tape (frequency response, d.c. 2,500 Hz). The output of the channel containing the action potentials was fed to a pulse height discriminator and a counter-timer coupled with a digital-analog converter, the output from which was plotted on a rectilinear recorder. The pulse height discriminator was used also in conjunction with another counter unit (this one having variable time gates), in order to count the number of baroreceptor impulses between successive R waves of the ECG. The counter output was recorded as a 'staircase', each step representing one nerve impulse. A femoral artery was cannulated and connected to a pressure transducer and a record of arterial pressure obtained on the pen recorder and on tape. ECG was also recorded on tape. The data stored on tape were later played back through the oscilloscope and photographed. The rectal temperature of the cats was monitored and maintained at 37°C by use of a heating pad placed beneath the animal.

To study the isolated and superfused carotid body, the preparation described by Eyzaguirre and Lewin [1961b] was employed. The superfusion fluid was Locke's solution with glucose (NaCl 6·0 g, KCl 0·42 g, $CaCl_2$ 0·24 g, glucose 1 g, Trizma base 6·0 g, normal HCl 39 ml., distilled water to 1 litre, pH 7·41 at 37°C) saturated with 100% O_2.

The prostaglandins used were PGE_2, PGA_2, and $PGF_{2\alpha}$-tromethamine salt (M.W. 475·6). Each mg of E_2 and A_2 was prepared as the sodium salt by dissolving it in 0·1 ml. ethanol with the subsequent addition of 0·9 ml. aqueous sodium chloride (0·9% w/v). The $PGF_{2\alpha}$ salt was water soluble but 0·1 ml. ethanol/mg was added. The stock solutions (1 mg/ml.) were kept frozen (−20°C) and working solutions of 20 μg/ml. prepared by diluting an aliquot of stock solution with Locke's solution. Prostaglandins were injected either intravenously or via the lingual catheter into the carotid artery over a period of 5–15 sec. An interval of 8 min was allowed to elapse between successive injections.

For the *in vitro* experiments prostaglandins were prepared in Locke's solution containing glucose to a final prostaglandin concentration of 10 μg/ml. This fluid, saturated with 100% O_2, was used to replace the Locke's solution which superfused the carotid body during control periods. The prostaglandin solutions superfused the preparation for up to 10 min. Prostaglandins (1 mg/ml.) were also applied locally

to the carotid body in the *in vitro* studies: 10 μl. (containing 10 μg) of solution could be applied directly onto the carotid body. NaCN (5 μg in 10 μl.) was also applied directly onto the carotid body in order to stimulate the chemoreceptors. For the *in vivo* studies, NaCN (5 μg) was injected into the carotid artery in order to stimulate chemoreceptor units.

Results

In vivo experiments

Records of electrical activity were obtained from carotid sinus nerves in five cats. Filaments containing either single or multiple active units were studied. The multi-unit recordings offer the advantage that they give a general indication of the pattern of response of the preparation. Separation of individual units was made by using the pulse height discriminator (see Methods). A total of eight baroreceptor filaments (three cats) and 15 chemoreceptor filaments (four cats) which contained only single or a few active units were studied, each fibre being recorded over a period of from 1 to 6 hr.

Baroreceptors

Baroreceptors were identified by their synchronous discharge with the arterial pressure oscillations [Heymans and Neil, 1958]. The prostaglandins were injected either intravenously or into the carotid artery in order to examine their effect on spontaneous baroreceptor activity.

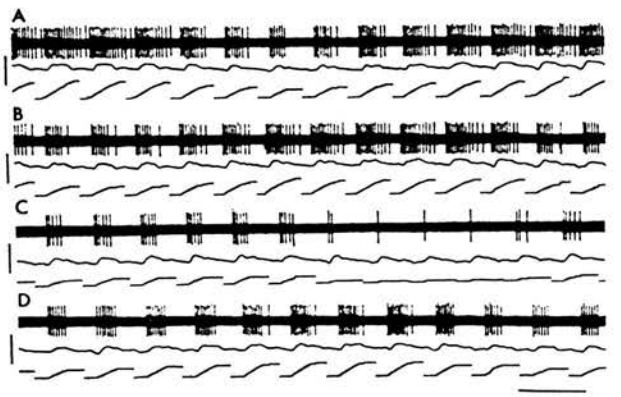

Fig. 1. Baroreceptor unit recorded from a filament of the carotid sinus nerve. The recording shows, from above downwards: nerve activity, blood pressure, number of impulses in each cardiac cycle. *A* is a control recording. *B* is 2·5 sec after the start of an injection of PGE_2 (8 μg) into the lingual artery. *C* and *D* are 30 sec and 3 min respectively after the injection. There is a respiratory fluctuation in blood pressure. The vertical bar is the blood pressure calibration (from 50 to 150 mm Hg) and the horizontal bar represents 1 sec.

PGE_2 (1·5–7·4 μg/kg i.v.; 0·7–3·0 μg/kg i.a.) evoked a dose-dependent reduction in the number of baroreceptor impulses per cardiac cycle when administered by either route. This reduction occurred with approximately the same latency, 15–20 sec, after onset of injection for both routes and was paralleled by a dose-dependent reduction in arterial blood pressure. When a correlation was made

between the onset of the pressure fall and the decrease in baroreceptor activity, it was clear that the decrease in baroreceptor activity followed beat to beat the reduction in pressure. Figs 1, 2 and Table I show the parallelism between arterial blood pressure changes and the alteration in baroreceptor firing sequence together with the absence of any direct effect of the prostaglandins on nervous activity (i.e. during the first 2–10 sec following close-arterial injection, see Fig. 1). Similar observations were made with PGA_2 (0·7–1·5 μg/kg i.v. or i.a.), the only difference being the lower doses required to elicit similar effects (PGA_2 being about 2–3 times more potent than PGE_2).

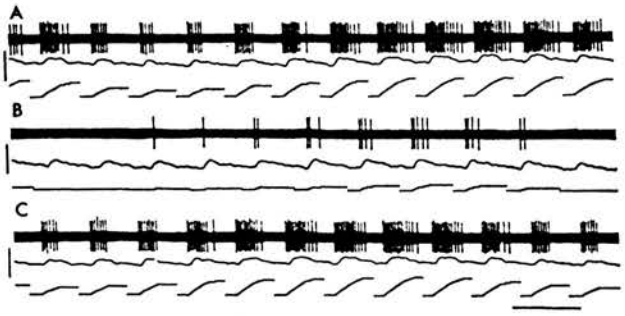

FIG. 2. Baroreceptor unit recorded from a filament of the carotid sinus nerve. Record details as for Fig. 1. *A* is a control recording. *B* is 25 sec after the i.v. injection of PGE_2 (8 μg) and *C* is 3 min later.

TABLE I. *Baroreceptor counts per second (c.p.s.) averaged over 10 sec from a single unit after injection of prostaglandin into the carotid artery (i.a.) or intravenously (i.v.). Blood pressure is in mm Hg.*

	Before prostaglandin		At time of maximal B.P. change		Three minutes after maximal B.P. change	
	B.P.	c.p.s.	B.P.	c.p.s.	B.P.	c.p.s.
PGE_2 4 μg i.a.	109	37	74	11	105	33
4 μg i.v.	100	30	68	9	105	32
PGA_2 2 μg i.a.	110	29	70	10	102	24
2 μg i.v.	91	25	55	6	111	29
$PGF_{2\alpha}$ 8 μg i.a.	115	37	105	27	114	36
8 μg i.v.	105	33	112	42	105	33

$PGF_{2\alpha}$ when injected i.a. (1·5–6·0 μg/kg) caused no constant changes in blood pressure or in baroreceptor discharge frequency. When administered i.v. (3·0–11 μg/kg) a transient rise in arterial pressure often occurred and this was associated with an increase in baroreceptor discharge (see Table I and Fig. 3). It appeared that the increased discharge frequency was secondary to the pressure rise because in animals where the blood pressure fell slightly, so did the discharge frequency.

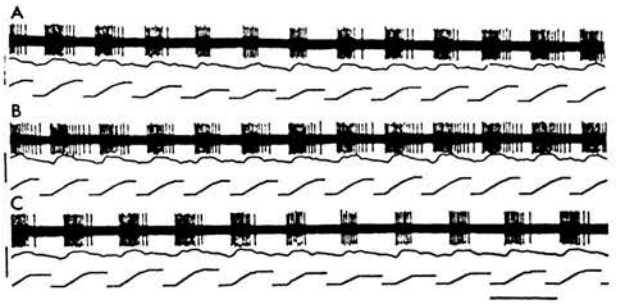

FIG. 3. Baroreceptor unit recorded from a filament of the carotid sinus nerve. Record details as for Fig. 1. *A* is a control period, *B* is 25 sec after the i.v. injection of $PGF_{2\alpha}$ (30 μg) and *C* is 3 min after the injection. There is a respiratory fluctuation in blood pressure.

Chemoreceptors

Chemoreceptor units were identified by their aperiodic pattern of discharge [Biscoe and Taylor, 1963] and their increase in impulse frequency with hypoxia or when NaCN was injected into the carotid artery.

PGE_2 (0·4–8·0 μg/kg) was injected into the carotid artery and the chemoreceptor discharge was reduced, this reduction occurring about 5 sec after the onset of the injection and before blood pressure changes were seen. Although the reduction in discharge frequency was dose-dependent, higher doses were associated with a shorter-lasting reduction which was sometimes followed by an increased chemoreceptor discharge and a fall in systemic blood pressure.

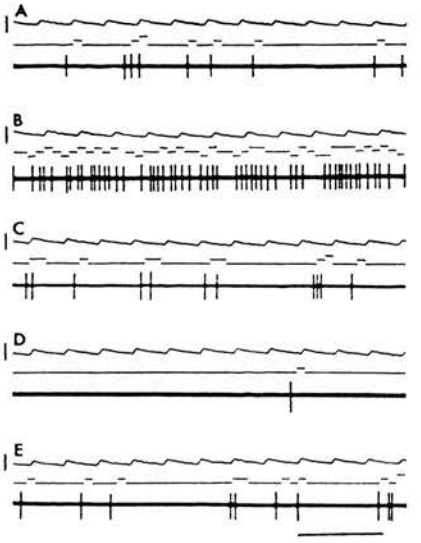

FIG. 4. Chemoreceptor unit recorded from a filament of the carotid sinus nerve. The recording shows, from above downwards: blood pressure, number of impulses in every 0·1 sec, nerve activity. *A* is a control period, *B* is 10 sec after the injection of sodium cyanide (10 μg) into the lingual artery. *C* is a further control period before the injection of PGE_2 (4 μg) into the lingual artery, *D*, and *E* is 3 min after the prostaglandin injection. Horizontal bar represents 1 sec and the vertical bar is the blood pressure calibration (from 50 to 150 mm Hg).

The reduction could be up to 90% of control values; it was not attributable to the injection volume because injection of the same volume of Locke's solution produced a transient reduction of chemoreceptor discharge seen only during the first 5 sec following injection (Fig. 4).

PGE_2 when injected i.v. (1·6–8·0 μg/kg) caused a dose-dependent increase in the chemoreceptor discharge with a latency of about 20 sec, the discharge frequency sometimes doubling. The increase in chemoreceptor discharge was paralleled by a fall in blood pressure, and the bigger the pressure reduction, the bigger was the increase in chemoreceptor discharge. Lowering the blood pressure by withdrawing venous blood caused an increase in chemoreceptor discharge (see Table II).

TABLE II. *Cat 1·9 kg. Increase in chemoreceptor discharge from a multiple chemoreceptor fibre in response to cumulative 2 ml. venous blood withdrawals. The discharge was averaged over 10 sec at the point when the systemic B.P. was lowest.*

Total blood withdrawn (ml.)	Mean B.P. (mm Hg)	Discharge (c.p.s.)
0	109	26
2	103	32
4	93	46
6	80	66
8	76	70
10	69	76

PGA_2 evoked responses similar to those seen with PGE_2, although lower doses were used (0·7–1·5 μg/kg i.v. or i.a.).

$PGF_{2\alpha}$ (1·5–8·0 μg/kg) injected into the carotid artery sometimes elicited a slight increase in chemoreceptor discharge, and at other times a slight decrease. These changes were not dose-dependent and were associated with small and variable changes in systemic blood pressure. $PGF_{2\alpha}$ (1·6–11 μg/kg) when injected i.v. caused either a small increase or decrease in blood pressure or no change at all. When the pressure fell the chemoreceptor discharge increased, and when the pressure rose the discharge decreased.

In vitro experiments

In order to establish whether the changes in chemoreceptor activity following prostaglandin administration were due to variations in carotid body blood flow, experiments were performed on five isolated and superfused carotid bodies from three cats. The chemoreceptor units exhibited a low frequency spontaneous discharge during superfusion with oxygenated Locke's solution and no change in discharge pattern could be detected during or after superfusion for 10 min with Locke solution containing either PGE_2 or $PGF_{2\alpha}$, 10 μg/ml.

Local application of 10 μg PGE_2 (10 μl.) to the carotid body did not affect the discharge, whereas 5 μg sodium cyanide (10 μl.) similarly administered before and after PGE_2 elicited an increase in discharge (Fig. 5).

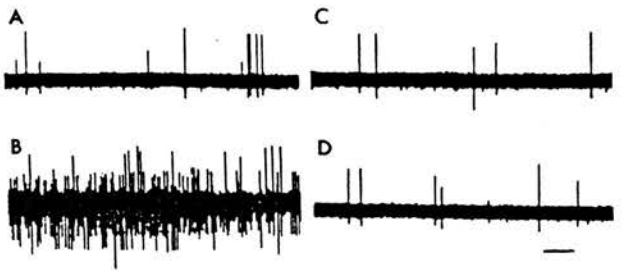

FIG. 5. Isolated superfused carotid body preparation from a 2·7 kg cat. The record shows nerve activity recorded from a filament of the carotid sinus nerve. *A* and *C* are control recordings and *B* shows the response 10 sec after the injection of sodium cyanide (5 μg) onto the carotid body. *D* is the response 60 sec after the injection of PGE_2 (10 μg) on to the carotid body. Horizontal bar represents 1 sec.

DISCUSSION

Our results suggest that PGE_2, PGA_2 and $PGF_{2\alpha}$, at the doses used, do not affect directly the baroreceptor activity of the carotid sinus, and that the observed changes in impulse frequency are secondary to variations in arterial pressure elicited by these substances. If the pressure changes resulted from a reflex initiated by a direct action of the prostaglandin on the sensory mechanism, then changes in baroreceptor activity should have preceded the pressure changes, but this was not the case.

In our *in vivo* experiments the ganglio-glomerular nerves were sectioned in order to avoid prostaglandin-induced changes in sympathetic activity altering the chemoreceptor discharge frequency. The sympathetic supply to the cat carotid body exerts only a slight influence on chemoreceptor activity during normal conditions [Floyd and Neil, 1952; Eyzaguirre and Lewin, 1961c] so it is not possible to say whether flow through the carotid body was affected by sympathetic denervation—one might predict a slight increase in flow. During hypotension an active sympathetic supply to the carotid body increases chemoreceptor activity [Floyd and Neil, 1952] and so our preparation would be expected to show less chemoreceptor activity during hypotension.

Changes in systemic blood pressure are accompanied by changes in carotid body blood flow in the cat [Daly, Lambertsen and Schweitzer, 1954] and it has been shown that hypotension in cats elicits a marked increase in chemoreceptor discharge [Landgren and Neil, 1951; Floyd and Neil, 1952]. Biscoe, Bradley and Purves [1970] used a perfused carotid sinus preparation and showed that lowering the perfusion pressure evoked an increase in chemoreceptor discharge, but that this was transient. We have found (see Table II), in agreement with Eyzaguirre and Lewin [1961a], that in cats with ganglio-glomerular nerves sectioned hypotension causes an increase in chemoreceptor discharge.

Administration of prostaglandins in the whole animal led to a change in chemoreceptor activity, but such alterations could not be obtained *in vitro*. The superfused carotid body preparation has been used to determine whether effects on chemoreceptor activity seen *in vivo* are secondary to vascular changes

[Zapata, Hess, Bliss and Eyzaguirre, 1969]. Our observations suggest that the variations in chemosensory activity elicited by the prostaglandins in the whole animal are evoked by changes in the carotid body blood flow.

PGE_2 is slightly less potent than PGE_1 in evoking respiratory and vascular effects in cats, but both are qualitatively similar [McQueen, 1972; unpublished observations]. PGE_2 and PGA_2 dilate systemic vessels [Horton, 1969] and such an action could explain the decrease in chemosensory activity produced by close arterial injection; blood flow to the carotid body would be increased. On the other hand, when a more general vasodilatation is obtained, as occurs on i.v. administration, an increase in chemoreceptor activity, secondary to the reduction in blood flow that accompanies the decrease in arterial pressure, could be expected.

$PGF_{2\alpha}$ caused variable changes in blood pressure, in some animals a rise in pressure being observed, in others a fall. The effect obtained depended upon the route of injection and the dose injected as well as on the particular animal; the reason why $PGF_{2\alpha}$ evoked different responses was not investigated. The impression that the alterations in chemoreceptor and baroreceptor discharge frequency were secondary to vascular effects was confirmed by the results from *in vitro* experiments.

Thus, our results suggest that prostaglandins do not affect directly either the baroreceptors or the chemoreceptors of the carotid region, and so appear contradictory to those of Kaplan *et al.* [1969]. The difference in experimental species studied could be advanced as a possible explanation for the lack of agreement. However, by a different interpretation of the data it is possible to reconcile our observations. Kaplan *et al.* evidently had high chemoreceptor activity in their preparations, because sinus denervation resulted in a fall in mean blood pressure (see their Figs 2 and 3, also Table I) instead of the increase that usually occurs after bilateral carotid nerve sectioning (Heymans and Neil, 1958). Increased chemoreceptor activity in dogs leads to increased sympathetic tone to the blood vessels and a blood pressure rise (McQueen and Ungar, 1971). If our evidence that prostaglandins do not affect the baroreceptors directly is accepted, then the fall in blood pressure in the experiments of Kaplan *et al.* can be explained by the reduction in chemoreceptor input to the central nervous system following the close arterial injection of PGE_1 due to dilatation of carotid body blood vessels resulting in increased blood flow through the carotid body. This effect would not be seen after denervating the carotid sinus region.

To conclude, we have been unable to demonstrate in our preparations any direct action of the prostaglandins, at the concentrations studied, on the baroreceptors and chemoreceptors of the carotid region. Modification of chemoreceptor activity is observed following prostaglandin administration, but this appears to be associated with vascular effects, both locally within the carotid body and systemically.

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The effects of ouabain on carotid chemoreceptor activity in the cat

By D. S. McQueen and J. A. Ribeiro. *Department of Pharmacology, University of Edinburgh, Edinburgh E118 9JZ*

It is not clear whether ouabain increases (e.g. McLain, 1970) or decreases (Joels & Neil, 1968) chemoreceptor activity, so the present study was undertaken to investigate further its effects on the cat carotid chemoreceptors.

Experiments were performed on pentobarbitone-anaesthetized cats which were artificially ventilated and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve and quantified (McQueen, 1977); the ganglioglomerular (sympathetic) nerves were left intact. Drugs were dissolved in Locke solution and either injected or infused into the ipsilateral common carotid artery (I.C.).

Ouabain was given either as successive injections (0·625, 1·25, 2·5, 5, 10, 20, 40, 80 μg in volumes of 0·1 ml. I.C., every 20–25 min) or as a continuous infusion (3 μg in 0·1 ml. min^{-1} I.C.). Its effect could be divided into two components, an initial excitation followed by a decrease in chemoreceptor activity. *Injections* of ouabain caused a dose-related increase in discharge lasting 1–2 min and a gradual increase in spontaneous discharge. When about 80 μg had been injected spontaneous discharge began to decrease, sometimes very rapidly, and further injections of ouabain were less effective; ultimately discharge ceased entirely. *Infusions* of ouabain caused a gradual increase in discharge frequency until about 20–30 μg had been infused when discharge began to decrease, eventually ceasing. The depression lasted for at least 2–3 hr after stopping the infusion.

Responses to the stimulants NaCN (5 μg I.C. or 50 μg I.V.), CO_2 (0·3 ml. I.C. CO_2-saturated Locke), acetylcholine (ACh, 50 μg I.C.) and to the inhibitor, dopamine (5 μg I.C.), were determined after injections and during infusions of ouabain. Responses to NaCN, CO_2 and ACh increased during the early excitatory period and were greatly reduced during the later period when discharge was decreasing. The effect of dopamine persisted during the excitatory period and was potentiated during the period of reduced discharge.

These results indicate that ouabain can both increase and then reduce the activity of the carotid chemoreceptors. During the excitatory phase chemoreceptor sensitivity to stimulants is increased, whereas during the later phase of decreasing discharge sensitivity to stimulants is reduced and that to inhibitors is increased.

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Excitatory action of adenosine on cat carotid chemoreceptors

By D. S. McQueen and J. A. Ribeiro. *Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ*

There do not appear to be any reports concerning the action of adenosine on cat carotid chemoreceptors. In view of the suggestions that adenosine may play a role in neurotransmission (e.g. Ribeiro, 1979), it seemed of interest to study the effect of adenosine on cat carotid chemosensory activity.

Experiments were performed on pentobarbitone-anaesthetized cats (42 mg kg^{-1} I.P. supplemented every 1–2 h). The animals were artificially ventilated with air and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve (McQueen, 1977); the ganglio-glomerular (sympathetic) nerves were usually cut. Drug solutions were injected into the ipsilateral common carotid artery (I.C.) over a 2 s period.

Adenosine (0·01–100 μg I.C.) caused a rapid and marked increase of spontaneous chemoreceptor discharge (e.g. Fig. 1), the intensity, duration and onset of which was dose-dependent. Theophylline (0·1–1 mg I.C.), an adenosine antagonist (see Fredholm, 1980), decreased spontaneous chemoreceptor discharge but did not significantly alter the excitatory action of adenosine.

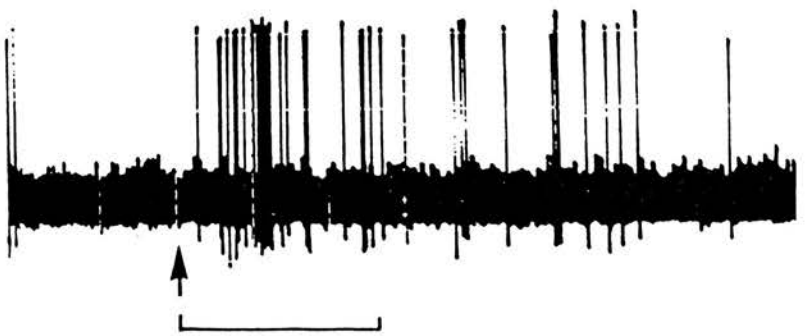

Fig. 1. Recording of a single chemoreceptor unit illustrating the response to adenosine (1 μg I.C.). Panel shows from above downwards: action potentials, injection marker and 10 s calibration.

Responses to the stimulants NaCN (2·5 or 5 μg I.C.), CO_2 (0·3 ml I.C. CO_2- saturated Locke), acetylcholine (ACh, 50 μg I.C.) and to the inhibitor, dopamine (DA, 5 μg I.C.), were determined before and after adenosine injections, and expressed as $\Delta\Sigma x$ (McQueen, 1977). Responses to NaCN and CO_2 were slightly and variably decreased after adenosine whereas both the excitatory response to ACh and the inhibitory response to DA were increased.

These results indicate that adenosine increases spontaneous chemoreceptor activity and facilitates the action of both ACh and DA on the carotid chemoreceptors.

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EFFECT OF ADENOSINE ON CAROTID CHEMORECEPTOR ACTIVITY IN THE CAT

D.S. McQUEEN & J.A. RIBEIRO[1]

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

1 The effects of intracarotid (i.c.) injections or infusions of adenosine on chemoreceptor activity recorded from the peripheral end of a sectioned carotid sinus nerve have been studied in cats anaesthetized with pentobarbitone.

2 Adenosine injections (0.1–100 μg) caused a rapid and marked increase of spontaneous chemoreceptor discharge, the intensity, duration and onset of which was dose-dependent. Infusion of adenosine, 50 μg/min, also evoked an increase in discharge which persisted for the duration of the infusion.

3 Both theophylline (1 mg i.c.) and aminophylline (1 mg i.c.) caused short-lasting decreases in spontaneous discharge but did not prevent the excitatory effect of adenosine. Theophylline increased the excitatory action of adenosine.

4 Naloxone (400 μg i.c.) antagonized the depressant effect of morphine on chemoreceptor discharge but not the excitatory action of adenosine.

5 It is concluded that exogenous adenosine can excite the cat carotid chemoreceptors, an effect which is not prevented by theophylline in the doses studied. The physiological significance of the findings is discussed.

Introduction

There do not appear to be any reports concerning the action of adenosine on peripheral arterial chemoreceptors. In view of the suggestions that adenosine may play a role in neurotransmission (e.g. Ribeiro, 1979), it seemed of interest to study the effect of adenosine on cat carotid chemosensory activity. A preliminary account of some of this work has been presented to the Physiological Society (McQueen & Ribeiro, 1981b).

Methods

Experiments were performed on 20 cats of either sex weighing between 2.4 and 3.4 kg (median 3.1 kg). The animals were anaesthetized with pentobarbitone sodium (42 mg/kg i.p. initially, supplemented by i.v. administration of 10% of the initial dose every 1 to 2 h), artificially ventilated and paralysed by gallamine triethiodide (3 mg/kg i.v.). The ganglioglomerular (sympathetic) nerves were usually cut. Full details of the experimental techniques, including the recording of arterial blood pressure and monitoring and regulation of arterial blood gas tensions and pH, have been given previously (McQueen, 1977; Docherty & McQueen, 1978).

[1] Permanent address: Centro de Biologia, Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Electrical activity of chemoreceptor units (1 to 6 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, passed through a pulse height (window) discriminator, and quantified with the aid of a PDP-8 computer.

A catheter was introduced via the lingual artery into the common carotid artery ipsilateral to the sinus nerve from which activity was recorded and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. In some experiments a second catheter was positioned in the same common carotid artery, this time via the superior thyroid artery, and used for infusing drug solutions (0.1 ml/min).

Drugs were dissolved in modified Locke solution (McQueen, 1977) or 0.9% w/v aqueous sodium chloride (saline). Drug solutions (0.1 ml, except adenosine 100 μg which was in 0.2 ml) were injected into the common carotid artery and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; injections were made over 2 s.

Results obtained from different experiments were pooled and expressed as the mean ± s.e. mean of the absolute values, which varied from experiment to experiment according to the number of units recorded. In order to determine whether changes observed were statistically significant, particularly when such changes were small, responses to particular drug

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doses in the different experiments were compared with the corresponding responses to the drug vehicle in the same experiments using either the Wilcoxon signed ranks test (when the number of pairs >7) or Student's paired t test (for <6 pairs, and assuming Gaussian distribution). The null hypothesis was rejected if $P<0.05$ and the difference between groups was considered statistically significant.

Drugs used were: adenosine, theophylline, aminophylline (Sigma); morphine sulphate, pentobarbitone sodium, gallamine triethiodide (May & Baker); naloxone hydrochloride (Endo).

Results

Injections of adenosine

The effects of intracarotid injection of different doses of adenosine on spontaneous chemoreceptor discharge in one experiment are illustrated in Figure 1. Discharge for each test was averaged over 10 s periods starting 10 s before and continuing for 60 s after the injection. As can be seen from either the neurograms or the averaged discharge, the main effect of adenosine was a dose-dependent increase in the frequency of discharge. No tachyphylaxis to the action of adenosine was observed. Thus, when injected at intervals of 5 min over a period of 1 h, there was no diminution of the effect on spontaneous chemoreceptor discharge.

Low doses of adenosine (0.001 to 0.01 μg) caused a decrease in chemoreceptor discharge during the first 10 s following the injection (see Figure 1), an effect which appeared to be most pronounced for the lowest dose. However, an inhibition of spontaneous discharge was also usually associated with injection of the same volume of drug vehicle, Locke solution

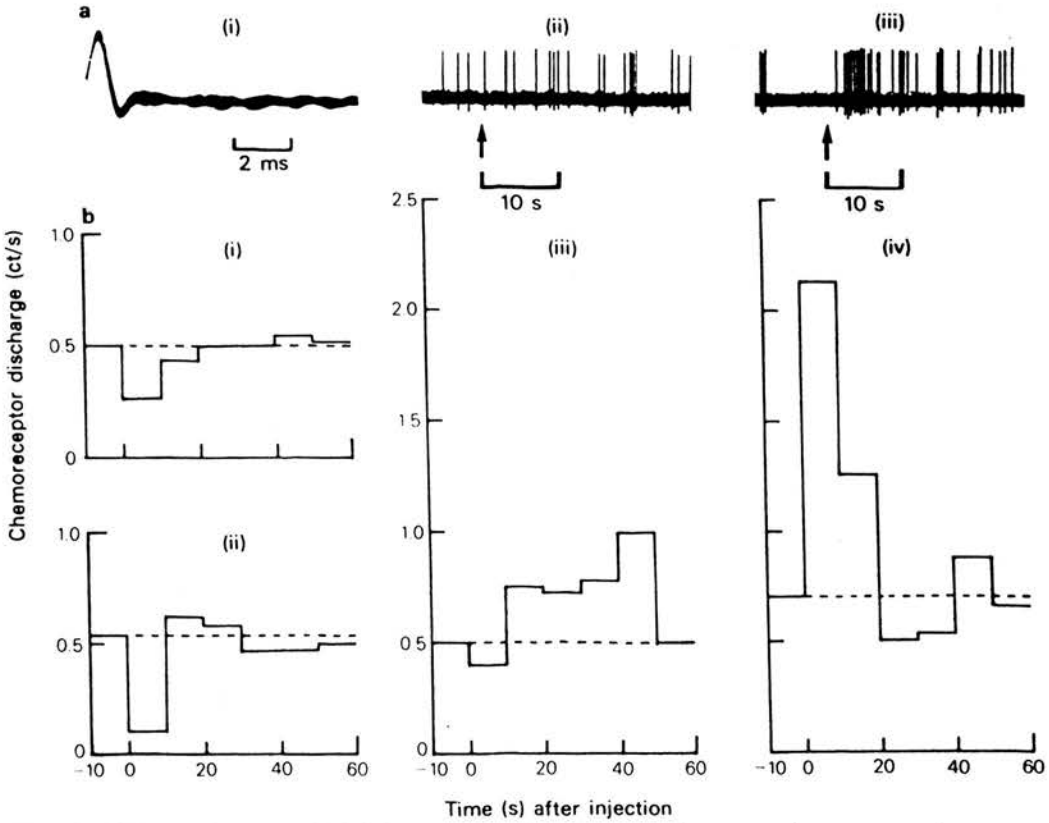

Figure 1 Effects of intracarotid (i.c.) injections of adenosine on the frequency of spontaneous chemoreceptor discharge. (a) Neurograms taken from one experiment show the increase in the discharge of a single unit (insert, i) caused by injecting (arrow) adenosine 0.1 (ii) and 1 μg (iii); in (i) the duration of the action potential is shown, the trace being of 10 consecutive superimposed spikes. (b) Responses of the chemoreceptor unit to i.c. injection of (i) 0.3 ml of the Locke solution and to adenosine: 0.001 μg (ii); 0.1 μg (iii) and 1 μg (iv). The graphs show the amplitude and duration of the responses averaged over 10 s periods following the injections.

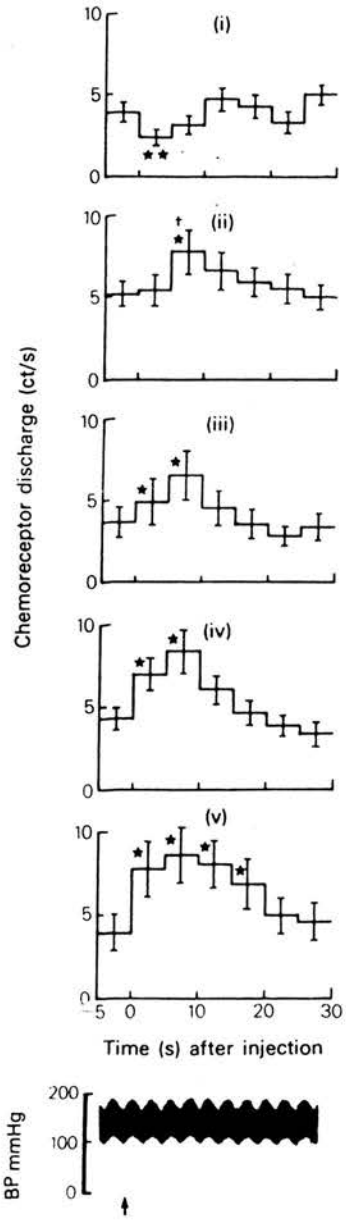

Figure 2 Effects on spontaneous chemoreceptor discharge of injecting Locke solution 0.3 ml (i) and adenosine 0.1 μg (ii); 1 μg (iii); 10 μg (iv) and 100 μg (v). Discharge was averaged over 5 s periods following the injection. Data obtained from tests in 8–10 cats were pooled and presented as the mean with vertical bars indicating s.e.mean. The lower panel shows the effect on arterial blood pressure (BP) of injecting (arrow) 100 μg of adenosine in one experiment. $*P<0.05$ compared with Locke solution injections; $**P<0.05$ compared with pre-injection control discharge; $\dagger P>0.05$ compared with adenosine 100 μg.

(see Figures 1 and 2), so responses to the low doses of adenosine were studied in five cats (results not shown) and compared with responses to injections of Locke solution (0.3 ml). There was no significant difference ($P>0.05$) between the responses to adenosine and Locke solution in these experiments.

Data obtained from ten experiments were averaged over 5 s periods for 30 s after the injection, pooled and plotted against time (Figure 2). This quantitative evidence confirmed that the increase in both amplitude and duration of the discharge were dose-related. The peak of the discharge, averaged over 5 s periods, for all doses (0.1–100 μg) occurred between 5 and 10 s after the injections, and that caused by 0.1 μg was not significantly different from that caused by the highest dose studied, 100 μg ($P>0.05$, $n=8$ pairs). Injections of Locke solution were also made in these experiments and the results obtained are summarized in Figure 2. They confirm the transient inhibitory effect of Locke solution illustrated in Figure 1. A significant decrease in discharge occurred during the first 5 s following the injection ($P<0.05$, $n=10$, compared with the pre-injection averaged discharge). This was less marked and not statistically significant in the next 5 s ($P>0.05$), and thereafter discharge returned to the pre-injection control levels.

Results from four experiments in which both average discharge and maximum discharge, expressed as ct/s, were obtained for different doses of adenosine are compared and summarized in Table 1. The average spontaneous chemoreceptor discharge was determined in the 15 s pre-injection control period immediately preceding each injection and in the 15 s post-control period commencing 45 s after the injections. Responses to adenosine were evaluated by determining the average discharge in the period during which discharge was increased above control frequency. Apart from increasing the average discharge, adenosine also increased the maximum discharge frequency. This latter increase, however, was not so marked as that averaged over the whole period, showing that the overall increase in discharge caused by adenosine resulted from a sustained increase throughout the response rather than from a sudden transient increase, such as occurs with acetylcholine (ACh) (cf. McQueen, 1977). The duration of the adenosine response was dose-dependent, and the delay to onset of the response was inversely related to dose.

The effects obtained were not related to changes in systemic blood pressure since adenosine in the doses studied (0.001 to 100 μg) did not cause any consistent or substantial changes in arterial blood pressure, as can be seen from Figure 2. There were no changes in arterial blood gas tensions or pH following administration of adenosine.

Table 1 Effect of adenosine on chemoreceptor discharge in the cat

	Chemoreceptor discharge						Response	
		Average discharge			Maximum discharge			
Adenosine (μg) (No. of expts)	Pre-control* (= 100%) (ct/s) Mean, range	Effect of adenosine (% of control) Mean, range	Post-control** (= 100%) (ct/s) Mean, range	Pre-control† (= 100% (ct/s) Mean, range	Effect of adenosine (% of control) Mean, range	Post-control‡ (= 100%) (ct/s) Mean, range	Duration (s) Mean, range	Delay To onset (s) Mean, range
0.1 (4)	6.1 5.2–6.9	156 108–184	5.1 3.9–5.8	12 11–14	135 73–181	11 8–13	8.0 6.0–11.7	7.1 3.2–10.1
1 (3)	4.9 3.6–6.2	181 172–191	5.0 3.7–7.1	10 8–12	142 70–206	11 8–12	12.7 9.0–15.4	5.4 2.1–11.3
10 (4)	4.0 1.9–5.1	209 195–218	3.6 1.3–4.7	9 5–12	175 143–196	10 6–13	13.8 12.1–14.6	4.7 2.1– 8.2
50 (3)	3.6 1.9–5.1	248 247–249	3.8 2.1–4.7	7 5–10	209 183–223	9 6–11	15.1 13.7–16.5	3.6 0.7– 8.4
100 (3)	5.5 1.8–7.6	229 221–243	5.5 2.8–6.9	13 5–17	209 180–240	12 7–15	19.8 14.3–25.4	1.7 0.5– 3.0

*Discharge averaged during the 15 s immediately before injecting adenosine.
**Discharge averaged during the 15 s period commencing 45 s after the injection, by which time the discharge had usually returned to pre-injection levels. There was no significant difference between pre- and post-control values ($P > 0.05$).
† Maximum discharge in 1 s observed during the pre-control period.
‡ Maximum discharge in 1 s observed during the post-control period.

Theophylline and aminophylline

Theophylline (1 mg) was given as an intracarotid (i.c.) injection in five cats and caused a decrease in spontaneous chemoreceptor discharge, an effect which lasted for about 30 s. The integrated response ($\Delta\Sigma x$) showed that the depression was significantly greater ($P < 0.05$) than that caused by injecting Locke solution. Although theophylline transiently decreased spontaneous chemoreceptor discharge (Figure 3), it did not prevent the excitatory action of adenosine, and as can be seen in Figure 4, the log dose-response curve to adenosine was shifted upwards and to the left which is indicative of an increase in the responses after theophylline; the response to adenosine 100 μg was statistically significantly greater after theophylline than it was before ($P < 0.05$, $n = 4$).

Injections of lower doses of theophylline (100–200 μg) did not cause any significant effect on the spontaneous discharge ($P > 0.05$, $n = 3$ pairs), and the excitatory effect of adenosine (1–100 μg) was unaltered by these doses ($P > 0.05$, $n = 3$ pairs).

Aminophylline (1 mg i.c.) was injected in one cat

Figure 3 Effect on spontaneous chemoreceptor discharge of injecting theophylline 1 mg i.c. (heavy line, ——) compared with that of injecting Locke solution (0.3 ml) (thin line, ——). Discharge was averaged over 10 s periods following the injection. * $P < 0.05$, $n = 5$ pairs. The upper panel shows the effect on arterial blood pressure of injecting (arrow) 1 mg of theophylline in one experiment.

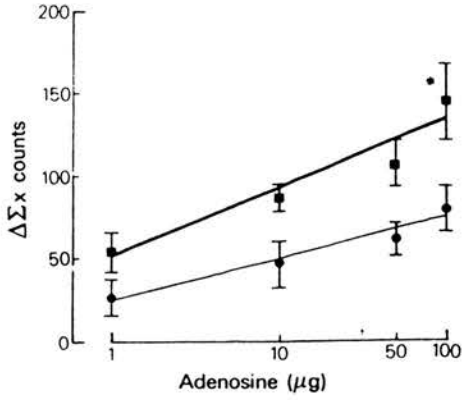

Figure 4 Dose-response data for adenosine obtained before (●) and after (■) theophylline (1 mg i.c.) in four experiments. Doses (μg i.c.) are plotted on a $\log_{10}$ scale and chemoreceptor responses expressed as the mean $\Delta\Sigma x$ for the four experiments; vertical lines show s.e.mean. $\Delta\Sigma x$ was calculated as the response during the 30 s following the injections. ($\Delta\Sigma x = \Sigma x - \bar{x}.t$, where Σx is the number of action potentials counted during the response of duration t s, a 'response' being defined as lasting from the first substantial change from background discharge frequency ($\bar{x}$ ct/s) until return to background level.) Lines were fitted to the data by the method of least squares. Averaged values ± s.e.mean (ct/s) for the pre-injection (15 s) control periods are as follows: adenosine 1 μg 4.5 ± 1.0 before theophylline, 6.5 ± 1.4 after theophylline; adenosine 10 μg 5.5 ± 1.4 before, 7.8 ± 1.7 after, adenosine 50 μg 3.5 ± 1.1 before, 5.2 ± 1.4 after; adenosine 100 μg 3.9 ± 1.7 before, 5.1 ± 2.0 after. * $P < 0.05$.

and the effect of adenosine (1–100 μg) tested before and after the injection. A transient depressant effect similar to that seen with theophylline was observed, and the excitatory action of adenosine was also undiminished after aminophylline.

Neither theophylline (Figure 3) nor aminophylline caused any marked changes in blood pressure and the highest dose of adenosine did not alter arterial blood pressure when injected after theophylline.

Naloxone

Four experiments were performed to investigate the influence of naloxone (0.4 mg i.c.) on chemoreceptor responses to adenosine and morphine. The adenosine results were pooled and are shown in Figure 5. No statistically significant difference was detected between the pre- and post-naloxone response ($P > 0.05$). In two of these experiments morphine (10–100 μg i.c.) was injected before and after naloxone (Figure 5). A chemodepressant effect of morphine was obtained in accordance with previous observations (McQueen & Ribeiro, 1980). This depressant effect of morphine was converted to an excitatory one by naloxone.

Infusions of adenosine

Adenosine, infused at a rate of 50 μg/min for 2 min in two cats, caused a sustained increase in chemoreceptor discharge, which returned to pre-infusion control levels within 30 s of stopping the infusion (Figure 6). The peak response was significantly different from the control, whether this was taken as the pre-infusion discharge or the discharge averaged during an infusion of a Locke solution (0.1 ml/min). The infusion of Locke solution did not affect spontaneous discharge (Figure 6). Adenosine infusions were not

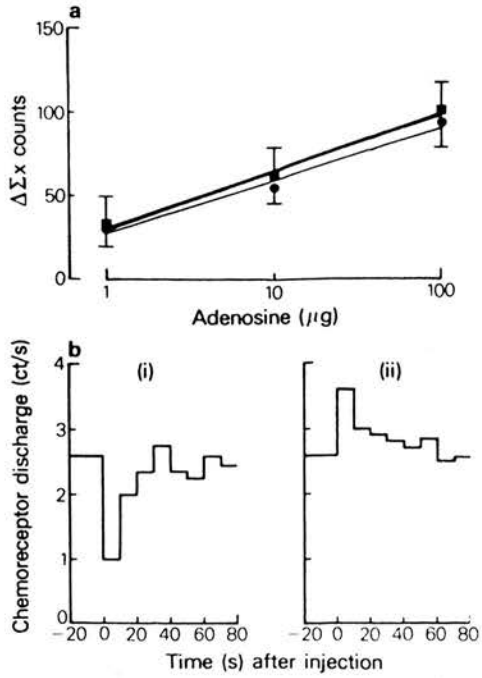

Figure 5 (a) Dose-response data for adenosine obtained before (●) and after (■) administration of naloxone (400 μg i.c.) in four experiments. Doses (μg i.c.) are plotted on a $\log_{10}$ scale and chemoreceptor responses expressed as the mean $\Delta\Sigma x$ for the four experiments; vertical lines show s.e.mean. $\Delta\Sigma x$ was calculated as the response during the 30 s following the injections. For details see Figure 4. Lines were fitted to the data by the method of least squares. Averaged values (ct/s) for the pre-injection (15 s) periods ± s.e.mean were as follows: adenosine 1 μg 4.5 ± 2.1 before naloxone, 5.7 ± 2.6 after naloxone; adenosine 10 μg 4.2 ± 1.4 before, 6.7 ± 1.5 after; adenosine 100 μg 6.5 ± 1.3 before, 5.0 ± 1.9 after. (b) Effects of morphine (100 μg i.c.) on chemoreceptor discharge before (i) and after (ii) naloxone (400 μg i.c.) in one experiment. Discharge was averaged over 10 s periods following the injection.

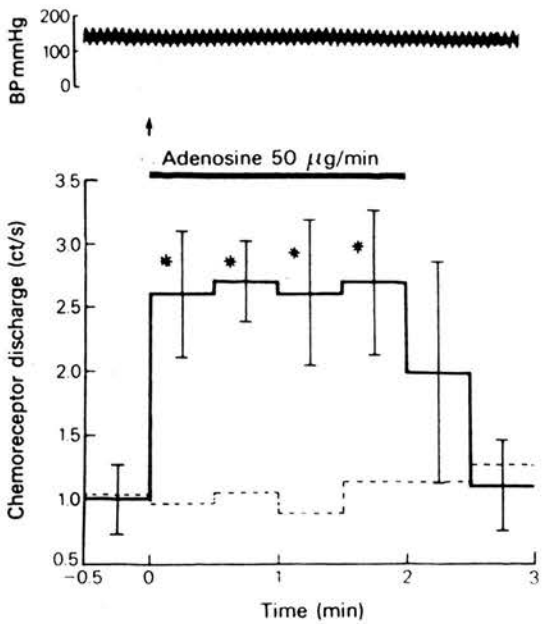

Figure 6 Pooled data from 6 infusions of adenosine (50 μg/min) in 2 cats showing the effect on spontaneous chemoreceptor discharge. The broken line represents the typical discharge during an infusion of Locke solution (0.1 ml/min). The upper panel shows a blood pressure trace recorded during one of the adenosine infusions. * $P < 0.05$.

associated with changes in arterial blood pressure, as can be seen from Figure 6.

Discussion

The results show that the predominant effect of injecting or infusing adenosine close-arterial to the cat carotid chemoreceptors is an increase in spontaneous discharge frequency.

In many preparations exogenous adenosine decreases the release of neurotransmitters (e.g. ACh (Ginsborg & Hirst, 1972; Ribeiro & Walker, 1975; Vizi & Knoll, 1976; Gustafsson, Hedqvist, Fredholm & Lundgren, 1978); noradrenaline (Hedqvist & Fredholm, 1976; Verhaeghe, Vanhoutte & Shepherd, 1977; Wakade & Wakade, 1978), dopamine (Michaelis, Michaelis & Myers, 1979) γ-aminobutyric acid (Hollins & Stone, 1978)). If adenosine exerts a similar action in the carotid body, its excitatory effect could be interpreted as being the consequence of decreased release of an inhibitory transmitter and/or modulator. Dopamine (Chiocchio, Biscardi & Tramezzani, 1966; Zapata, Hess, Bliss & Eyzaguirre, 1969) and enkephalins (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980) appear to be present in the cat carotid body and both exogenous dopamine (Zapata, 1975; Docherty & McQueen, 1978) and the enkephalins (McQueen & Ribeiro, 1980; 1981a; McQueen, 1981) depress spontaneous chemoreceptor activity. Osborne & Butler (1975) suggested that tonically released dopamine may suppress chemoreceptor discharge and although evidence which is not in agreement with their hypothesis has been obtained (e.g. Docherty & McQueen, 1978), the present results could be explained in terms of adenosine inhibiting the release of dopamine, or some other substance which is tonically active in inhibiting discharge (? enkephalins).

However, it has also been found that adenosine can excite nerve cells. For example, Siggins, Gruol, Padjen & Forman (1977) showed that adenosine depolarizes neurones of explanted amphibian sympathetic ganglia, and Bleehen & Keele (1977) described algogenic actions of adenosine on the human blister base. It appears that adenosine sensitizes the carotid chemoreceptors to ACh and dopamine (McQueen & Ribeiro, 1981b) which might be construed as evidence that adenosine acts directly on the postsynaptic component of the carotid body chemosensory synapse, i.e. on the sensory nerve ending. An adenylate cyclase-cyclic AMP system is apparently located in the sinus nerve endings (Fitzgerald, Rogus & Dehghani, 1977) and it may be that adenosine interacts with this in a manner similar to that described for the brain (e.g. Davies, Taylor, Gregson & Quinn, 1980). Further investigation is needed to explore this possibility.

Theophylline appears unable to prevent activation of P_2-purinoceptors. These receptors, as so far described, seem to be localized post-junctionally (see Burnstock, 1978). De Mey, Burnstock & Vanhoutte (1979) found that in the canine saphenous vein, theophylline antagonizes the inhibitory effect of ATP on neurogenic responses but not its direct contractile effect. According to these authors this suggests the presence of both inhibitory presynaptic (P_1) and excitatory postsynaptic (P_2) receptors. In the present study the excitatory effect of adenosine was unaffected by low doses of theophylline (0.1–0.2 mg) but potentiated by a slightly higher dose (1 mg), a finding that is compatible with a P_2-excitatory post-junctional effect. However, the potentiation might also have resulted from theophylline antagonizing a chemodepressant component of the adenosine response, which might normally be masked by the excitatory component, or from mechanisms having in common the ability to increase the cyclic AMP concentration (e.g. theophylline by inhibiting phosphodiesterases, and adenosine by activating the adenylate cyclase).

It has also been proposed that the inhibitory action of morphine may involve the release of adenosine as an intermediary (Sawynok & Jhamandas, 1976; Stone & Perkins, 1979), and it has been shown that morphine and enkephalins can inhibit spontaneous carotid chemosensory discharge in the cat (McQueen & Ribeiro, 1980; 1981a; McQueen, 1981). However, naloxone reduces the inhibitory effect of morphine without reducing the excitatory response to adenosine, so the effects of adenosine do not appear to result from actions on naloxone-sensitive opiate receptors in the carotid body.

Adenosine can cause vasodilatation (see Berne, Foley, Watkinson, Miller, Winn & Rubio, 1979) and we cannot, therefore, preclude the possibility that adenosine was changing blood flow in the carotid body and thereby, perhaps, altering chemoreceptor discharge, even though it had little overall effect on the systemic blood pressure. However, other vasodilators (e.g. sodium nitrite, sodium nitroprusside: Docherty, 1980) tend to cause a delayed decrease in discharge, which is the opposite of the main effect obtained following adenosine injections in the present experiments. We consider it unlikely that vascular effects are responsible for the greater part of the response to adenosine, but would wish to investigate this with *in vitro* carotid body studies.

Adenosine is released into the circulation by a number of physiological and pathophysiological processes (e.g. ischaemia; see Winn, Rubio & Berne, 1979), and a correlation between adenosine concentration and oxygen supply in rat brain has been suggested (Rubio, Berne, Bockman & Curnish, 1975). In the present study, low doses of adenosine modified chemoreceptor discharge and it seems reasonable to speculate that adenosine might influence events taking place in the carotid body, perhaps by modulating any putative transmitter(s) which may be released within the carotid body complex, and/or by directly activating sensory nerve endings.

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EFFECTS OF OUABAIN ON CAROTID BODY CHEMORECEPTOR ACTIVITY IN THE CAT

BY D. S. McQUEEN AND J. A. RIBEIRO*

From the Department of Pharmacology, Univeristy of Edinburgh Medical School, 1 George Square, Edinburgh EH8 9JZ

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SUMMARY

1. The effects of infusions of ouabain on chemoreceptor activity recorded from the peripheral end of a sectioned carotid sinus nerve were studied in cats anaesthetized with pentobarbitone.

2. Ouabain caused a marked increase in chemoreceptor discharge followed by a decline in discharge to frequencies near or below the pre-ouabain level; during the latter period further administration of ouabain had no effect.

3. Infusion of ouabain during hypoxia further increased the chemoreceptor discharge, but this effect was short-lasting.

4. On intracarotid administration ouabain was less effective in cats with the ganglioglomerular (sympathetic) nerves cut, whereas on intravenous administration no significant difference was observed. Following intravenous administration of ouabain the chemoreceptor peak discharge occurred with dose levels similar to those needed to cause cardiac arrhythmias, but following intracarotid administration the chemoreceptor discharge peaked at doses about 40% of those causing arrhythmias.

5. During ouabain-induced excitation the stimulatory action of NaCN, CO_2-equilibrated Locke solution and acetylcholine was potentiated, as was the chemo-inhibition induced by dopamine. During the post-excitatory period the responses evoked by these substances were reduced or abolished.

6. Neither mecamylamine, a nicotinic antagonist, nor physostigmine, an anti-cholinesterase, affected the response of the carotid chemoreceptors to ouabain.

7. The major finding of this study was that ouabain initially 'sensitizes' the carotid body chemoreceptors and then 'desensitizes' them. The most likely mechanism responsible for these effects is the well established Na^+–K^+-ATPase-inhibiting property of ouabain.

INTRODUCTION

There are several reports concerning the effects of ouabain, a cardiac glycoside, on carotid body chemoreceptor activity. For example, neural traffic recorded from the carotid sinus nerve is increased by ouabain (McLain, 1970), although this includes baroreceptor as well as chemoreceptor activity. Joels & Neil (1968) found that the increase in chemosensory discharge evoked by perfusing vascularly isolated cat

* Present address and correspondence: Instituto Gulbenkian de Ciência, 2781-Oeiras, Portugal.

carotid bodies with nitrogenated solutions is reduced by ouabain. Some, but not all carotid body type I cells *in vitro* are depolarized by ouabain (Eyzaguirre, Baron & Gallego, 1977).

We considered that, in order to establish the effects of ouabain on carotid chemoreceptors *in situ*, further experiments were needed. Accordingly, we performed a quantitative neuropharmacological investigation in cats; a preliminary report on some of the results from the study has been made to the Physiological Society (McQueen & Ribeiro, 1980).

METHODS

The experimental details have previously been described fully (McQueen, 1977) and only a brief summary follows. Experiments were performed on twenty-three cats of either sex weighing between 2·4 and 3·7 kg (mean 3·0 ± 0·1 kg), anaesthetized with pentobarbitone sodium (42 mg/kg I.P.) supplemented every 1·5–2 h by intravenous administration of 10 % of the initial dose. The animals were artificially ventilated with room air and paralysed with gallamine triethiodide (3 mg/kg I.V., supplemented every 1–1·5 h). Blood pressure was recorded from a femoral artery, and electrocardiogram monitored throughout the experiment. The bladder was drained at regular intervals and the rectal temperature maintained at 38 ± 0·5 °C. End-tidal CO_2 was continuously monitored by an infra-red CO_2 analyser (Med 1 A; Grubb Parsons) and the P_{a,CO_2}, P_{a,O_2} and pH of femoral arterial blood samples measured using a gas monitor (BMS 3 with PHM 71 meter, Radiometer).

Drug *injections* (0·1 ml) were made into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded, and washed in with 0·2 ml modified Locke solution which had been bubbled with 5 % CO_2/95 % air in a water bath at 37 °C. The injections were completed over a 2 s period commencing at the peak of the inspiratory phase of the respiratory cycle. In two cats successive injections of ouabain (1·25–160 μg I.C.) were made every 15–25 min. However, difficulties associated with interpreting the results from these pilot experiments led to the use of ouabain infusions as described in Results. Drug *infusions* were made through a second catheter situated in the common carotid artery at its junction with the superior thyroid artery (through which the catheter was introduced) using an infusion pump (0·1 ml/min; Braun Unita). The catheter dead-space was 0·2 ml.

A sinus nerve was cut and electrical activity from single or multiple (two to six) chemoreceptor units was recorded from filaments of the peripheral nerve using bipolar Pt–Ir electrodes. In thirteen cats the ganglioglomerular (sympathetic) nerves were cut. Exposed tissues were covered with warm (37 °C) mineral oil. Sensory nerve discharge was amplified by an a.c. amplifier (Neurolog), displayed on an oscilloscope and recorded on one channel of a tape-recorder (Tandberg 115; frequency response d.c. to 1250 Hz), and subsequently analysed with the aid of a microcomputer (Commodore 3032) in order to provide data concerning discharge frequency. The average ($\bar{x}$ counts/s) and total counts (Σx) were calculated from each response after its duration (t seconds) had been determined. Responses were expressed as a change from control level by subtracting the appropriate control or background discharge (i.e. as $\Delta\Sigma x$). Data from different experiments were pooled and the results presented as the mean ± S.E. of mean. When recording simultaneously from both carotid sinus nerves another a.c. amplifier and a second channel of the tape-recorder were used.

Statistical analysis

The significance of differences between means was calculated using Student's paired or unpaired t test; P values of 0·05 or less were considered to represent statistically significant differences.

Drugs

Drugs were prepared in modified Locke solution (McQueen, 1977). Doses referred to are those of the salts.

The drugs used were: sodium pentobarbitone, gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide, atropine sulphate (B.D.H.), dopamine hydrochloride (Koch Light) and ouabain octahydrate (Sigma).

RESULTS

A typical response of the carotid chemoreceptors to an intracarotid infusion of ouabain (3 μg/min) in a cat with intact ganglioglomerular nerves is shown in neurogram-form in Fig. 1, and graphically in Fig. 2. Results from seven experiments

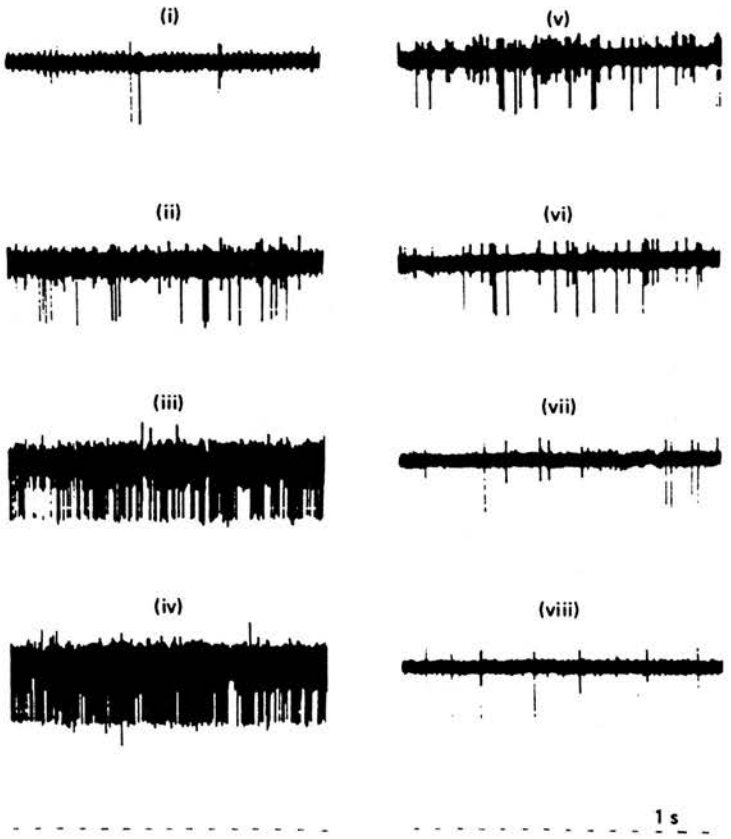

Fig. 1. Neurograms illustrating the effect on chemoreceptor discharge of an infusion of ouabain (3 μg/min I.C.) in a cat with intact ganglioglomerular nerves. (i) Pre-infusion (control) discharge; (ii) 10, (iii) 14, (iv) 16, (v) 25, (vi) 27, (vii) 30 min after starting an infusion; (viii) 1 min after stopping the infusion. The broken line at the bottom of the Figure is the time calibration in seconds.

in which ganglioglomerular nerves remained intact were pooled and are shown in Table 1 (p. 228). Before starting a ouabain infusion, NaCN (5 μg) was injected via the superior thyroid and lingual catheters and the chemoreceptor responses were compared, in order to determine whether catheter position had any appreciable effect on the amount of drug reaching the chemoreceptors. No significant differences were detected in relation to the discharge evoked or the duration of response; the mean time to onset of response was greater by 0·7 s for the thyroid catheter, though this was not statistically significant ($P > 0{\cdot}05$, paired t test).

Infusion of the drug vehicle (Locke solution, 0·1 ml/min) via the superior thyroid artery (control experiment) did not cause any appreciable changes in the frequency of spontaneous chemoreceptor discharge (Fig. 2). In contrast, an infusion of ouabain (3 μg/min I.C.) led to an increase in chemoreceptor discharge. Discharge increased slowly during the first 10 min of the infusion, as shown in Fig. 2; this initial slow

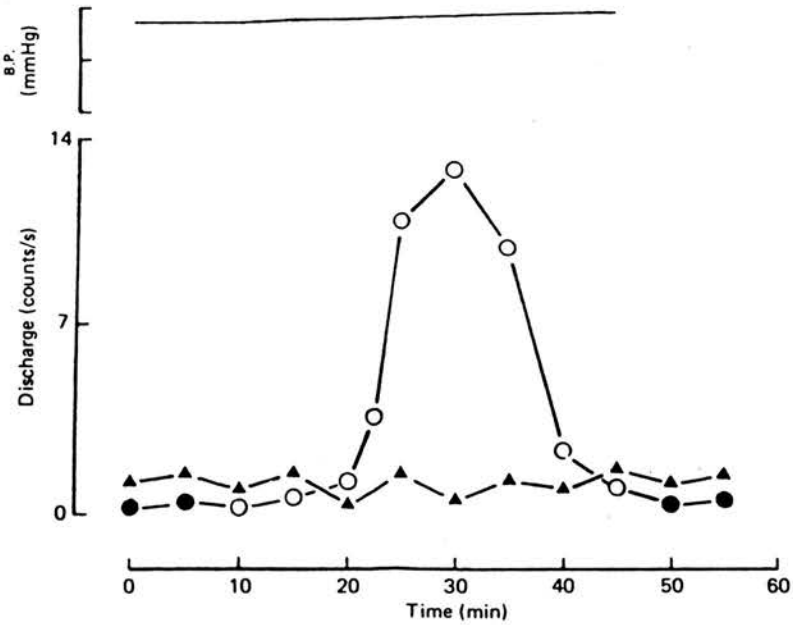

Fig. 2. Effect of a ouabain infusion (3 μg/min I.C.) on chemoreceptor discharge in a cat with intact ganglioglomerular nerves. The ordinates are the computed averages of the number of chemoreceptor action potentials. The abscissa shows the time at which the averaging began. ▲, Locke infusion (0·1 ml/min); ●, discharge before or after ouabain infusion; ○, during ouabain infusion. The upper part shows the mean femoral arterial blood pressure (B.P.) recorded concurrently. B.P. calibration 0–100–200 mmHg. The mean values (three determinations) for $P_{a.O_2}$, $P_{a.CO_2}$ and pH determined before ouabain infusion (at about the peak discharge) were: $P_{a.O_2}$ 12·5 kPa; $P_{a.CO_2}$ 4·2 kPa; pH 7·37. During ouabain infusion they were: $P_{a.O_2}$ 12·6 kPa; $P_{a.CO_2}$ 3·9 kPa; pH 7·34.

increase was consistent in all experiments. Thereafter, the discharge rose and 3–5 min later a sudden and marked increase occurred which was followed by a decline, with discharge returning to a level of the same order or slightly below that recorded before starting the ouabain infusion. The discharge remained at this reduced level after stopping the infusion. Chemoreceptor discharge was followed post mortem in five animals in which death had been caused by ouabain. During the 10–40 min after cardiac arrest the chemoreceptors continued to discharge, although the frequency tended to decay during these periods.

Fig. 2 also shows the mean femoral arterial blood pressure (B.P.). The over-all effect of ouabain was to cause an increase in mean B.P. which began to be apparent 5–10 min after starting the infusion. The averaged B.P. recorded at the time of peak discharge for the seven experiments was 175 ± 12 mmHg, whereas the control (obtained before

starting ouabain infusion) was 142±11 mmHg (see Table 1). The difference, 33±8·3 mmHg, was statistically significant ($P < 0·05$, paired t test).

Under the present experimental conditions (extracellular recordings) no substantial modification of either the amplitude or duration of the action potentials was detected during ouabain infusions. The effects of ouabain were not a consequence of changes

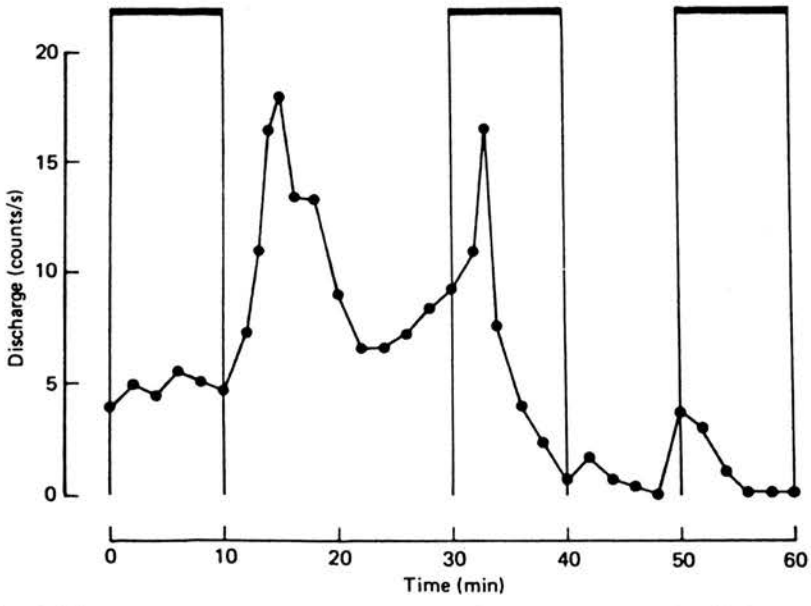

Fig. 3. Effect on spontaneous chemoreceptor discharge (counts/s) of three consecutive ouabain infusions (3 μg/min I.C.), during 10 min periods represented by the horizontal bars, in a cat with intact ganglioglomerular nerves. Recordings from two or three chemoreceptor units. Note the increase in the discharge after stopping the first ouabain infusion, the decrease in the intensity and duration of the excitation during the second infusion and the absence of effect during the third infusion.

in P_{a,O_2}, P_{a,CO_2} or pH of the blood since no substantial changes in these parameters occurred during ouabain infusions in samples taken at the peak of the chemoreceptor response. Values for P_{a,O_2}, P_{a,CO_2} and pH of the blood were as follows: P_{a,O_2} 12·0±0·4 kPa before and 12·1±0·3 kPa during ouabain infusion; P_{a,CO_2} 4·0±0·2 kPa before and 4·2±0·1 kPa during infusion; pH 7·34±0·02 before and 7·30±0·02 during infusion ($n = 7$).

The general pattern of chemoreceptor discharge was not markedly different when counting either single or multiple units (two to six) from multi-unit recordings, although in one experiment a single unit stopped discharging sooner than the other units, which may be an indication of differential sensitivity to ouabain.

In an attempt to test whether the action of ouabain on chemoreceptor activity could be related to a vascular mechanism, namely vasoconstriction caused by ouabain infusion (Treat, Ulano & Jacobson, 1971), two experiments were performed in which the ouabain infusion (3 μg/min I.C.) was stopped after 10 min. The results from one of these experiments are shown in Fig. 3. The excitatory action of ouabain occurred

after stopping the infusion, the effect being similar to that observed in experiments in which the ouabain infusion was continued. Fig. 3 also shows the effect of a further two infusions, each of 10 min duration, the first separated from the second by 20 min and the third from the second by 10 min. The second infusion induced a short-lasting increase in the chemoreceptor discharge and the third caused little or no effect on discharge. Following the last infusion no chemoreceptor responses were obtained to further ouabain infusions or to substances such as NaCN (5 μg I.C.), CO_2-equilibrated Locke solution (0·3 ml I.C.), acetylcholine (ACh: 50 μg I.C.) or dopamine (5 μg I.C.) (see section below on evoked responses during uninterrupted ouabain infusions).

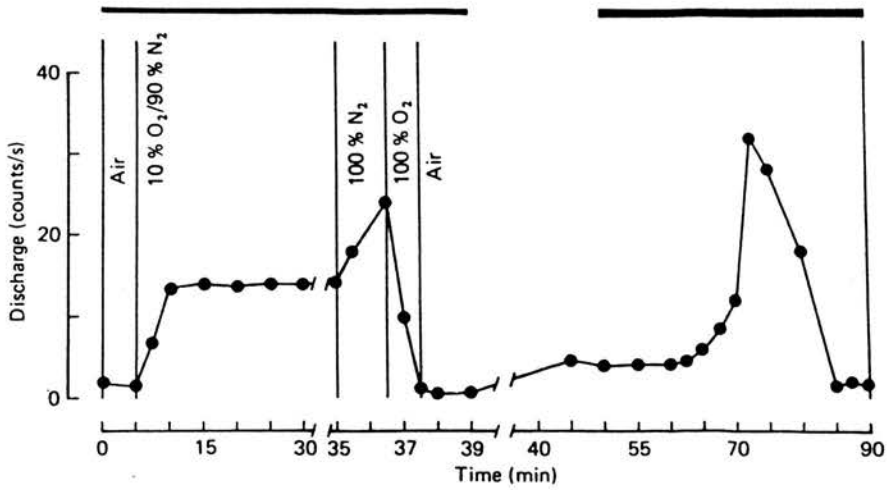

Fig. 4. Effect on chemoreceptor discharge of hypoxic stimulation (breathing 10% O_2/90% N_2), followed by 100% N_2, and then by hyperoxia (100% O_2) during an infusion of Locke solution (0·1 ml/min I.C.) in a cat with intact ganglioglomerular nerves. After discharge returned to a steady level on breathing air, ouabain (3 μg/min I.C.) was infused. The duration of the Locke infusion is indicated by the thin horizontal bar, that of the ouabain infusion by the thicker bar. Recordings obtained from one or two chemoreceptor units.

Comparison between the response of chemoreceptors to hypoxia and to ouabain

In order to compare the response of chemoreceptors to hypoxia with that to ouabain, an experiment was designed in which Locke solution was infused (0·1 ml/min I.C.) and the animal made hypoxic by breathing 10% O_2/90% N_2. As a result of this procedure peak chemoreceptor discharge occurred about 5 min after switching to the hypoxic gas mixture and discharge remained elevated throughout the period of hypoxic stimulation (30 min). Thereafter the hypoxic stimulus was intensified by ventilating the cat with 100% N_2 for 90 s and a further increase in the discharge was then observed (Fig. 4). On switching to breathing O_2 (100%) for the next 60 s chemoreceptor discharge returned to a level below that recorded before starting the hypoxic procedure. This protocol was an attempt to mimic the pattern of discharge observed during ouabain infusions. After spontaneous discharge returned to a steady level, which occurred after 10 min of air-breathing, an infusion of ouabain (3 μg/min)

was initiated. The glycoside caused an increase in discharge (Fig. 4) with the same pattern as that described previously (see e.g. Fig. 2).

The effect of ouabain during hypoxia

The effect of ouabain (3 μg/min infused I.C.) during hypoxic stimulation (animal breathing 15 % O_2/85 % N_2) is illustrated in Fig. 5. An infusion of Locke solution was used as control and the hypoxic stimulation applied in its presence. Within 5 min

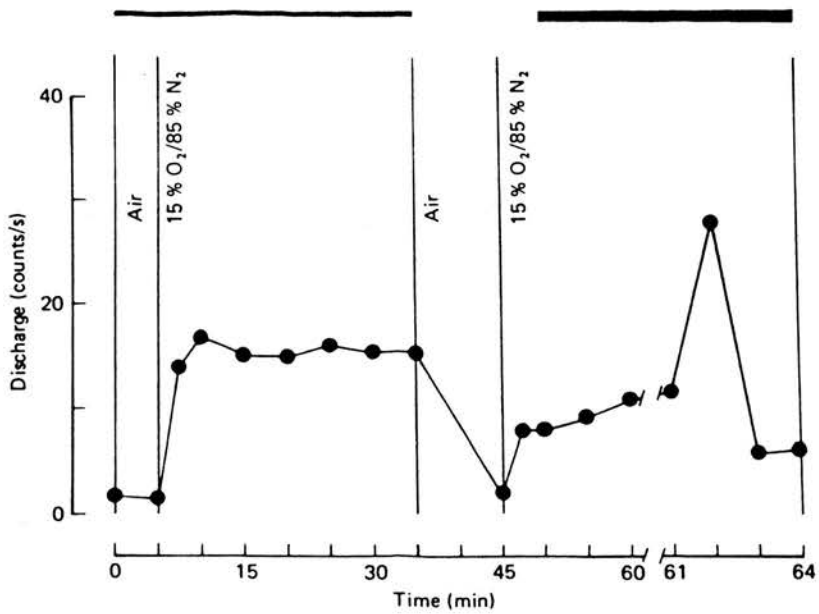

Fig. 5. Effect of a ouabain infusion (3 μg/min I.C.) on chemoreceptor discharge evoked by hypoxic stimulation (15 % O_2/85 % N_2) is illustrated in the right-hand side of the Figure. The left-hand side shows the effect of a hypoxic stimulus (15 % O_2/85 % N_2) on spontaneous chemoreceptor discharge in the presence of a Locke solution infusion (0·1 ml/min I.C.) in a cat with intact ganglioglomerular nerves. The duration of the Locke infusion is indicated by the thin horizontal bar, that of the ouabain infusion by the thicker bar. Recordings obtained from one or two chemoreceptor units.

of switching to the hypoxic gas mixture chemoreceptor discharge increased to a level which was sustained throughout the hypoxic stimulation period (30 min). Thereafter the discharge returned to the pre-hypoxia levels. The hypoxic stimulus was again applied, and when a steady chemoexcitatory response had been obtained an infusion of ouabain was begun. Under these conditions ouabain caused a slow increase in the discharge during the first 10 min after starting the infusion which was followed by a short-lasting peak (= 120 s), and a decay in chemoreceptor discharge to slightly below the pre-infusion level.

The influence of the ganglioglomerular (sympathetic) nerves on the effects of ouabain on chemoreceptors

Table 1 summarizes the results of investigations into the influences exerted by ganglioglomerular nerves on chemoreceptor responses to ouabain. Ouabain was

administered by either intravenous or intracarotid infusion. For each route of administration a comparison was made between cats with either intact or cut ganglioglomerular nerves. It is evident that the mean dose of ouabain required to increase chemoreceptor discharge maximally when the ganglioglomerular nerves were cut is significantly greater ($P < 0.05$) than in cats with the nerves intact (Table 1). Such a dose difference, however, was not detected when ouabain was infused intravenously and recordings obtained from intact and denervated (opposite side) carotid bodies of the same animal in three experiments (Table 1). The increase in discharge during intravenous infusion was, however, significantly greater on the side with intact ganglioglomerular nerves.

TABLE 1. Influence of the ganglioglomerular (sympathetic GG) nerves on the effects of ouabain on chemoreceptor discharge

Experimental conditions			Pre-ouabain control (counts/s)	Ouabain effect at the peak (counts/s)	Dose at the peak (μg/kg)	B.P. (mmHg)	
						Control	At the peak
Ouabain I.C.	Group 1,	7	3·1	17·2	18·7	142	175*
	GG intact		±1·1	±3·6	±1·8	±11	±12
	Group 2,	6	2·2	16·9	36·8**	141	179*
	GG cut		±0·8	±3·2	±3·5	±9	±10
Ouabain I.V.†	Left side,	3	5·0	55·0	67·9		
	GG intact		±0·5	±9·7	±11·3	165	219*
	Right side,		5·5	36·0***	65·6	±18	±17
	GG cut		±0·9	±11·4	±16·8		

Data expressed as mean ± S.E. of mean.
* $P < 0.05$, paired t test compared with control.
** $P < 0.05$, paired t test compared with group 1.
*** $P < 0.05$, t test compared with left side GG intact.

† Recordings from both carotid sinus nerves (left and right sides) were obtained concurrently. Ouabain was infused at the same rate as I.C. (3 μg/min I.V.). Lingual arteries of both sides were cannulated.

Cardiotoxic actions of ouabain

The doses of ouabain required to cause maximal chemoreceptor discharge are shown in Table 1. Comparing these doses with those needed to cause ventricular extrasystoles when administered by the intracarotid route (64 ± 5 μg/kg, $n = 3$) or intravenously (69 ± 2 μg/kg, $n = 6$: Peres-Gomes & Ribeiro, 1979) showed that with intracarotid ouabain a significant difference ($P < 0.05$) exists between chemoreceptor activation and cardiotoxic doses, whereas little or no difference is apparent between chemoreceptor activation and cardiotoxic effect when ouabain is administered intravenously.

Evoked responses

Intracarotid injections of NaCN (5 μg), CO_2-equilibrated Locke solution (0·3 ml), ACh (50 μg) and dopamine (5 μg) were made before and during ouabain infusions (3 μg/min I.C.). These doses evoked chemoreceptor responses which were supra-threshold but sub-maximal. In Fig. 6 the times at which injections of these substances were made are approximate, i.e. the injections were made at some time during the

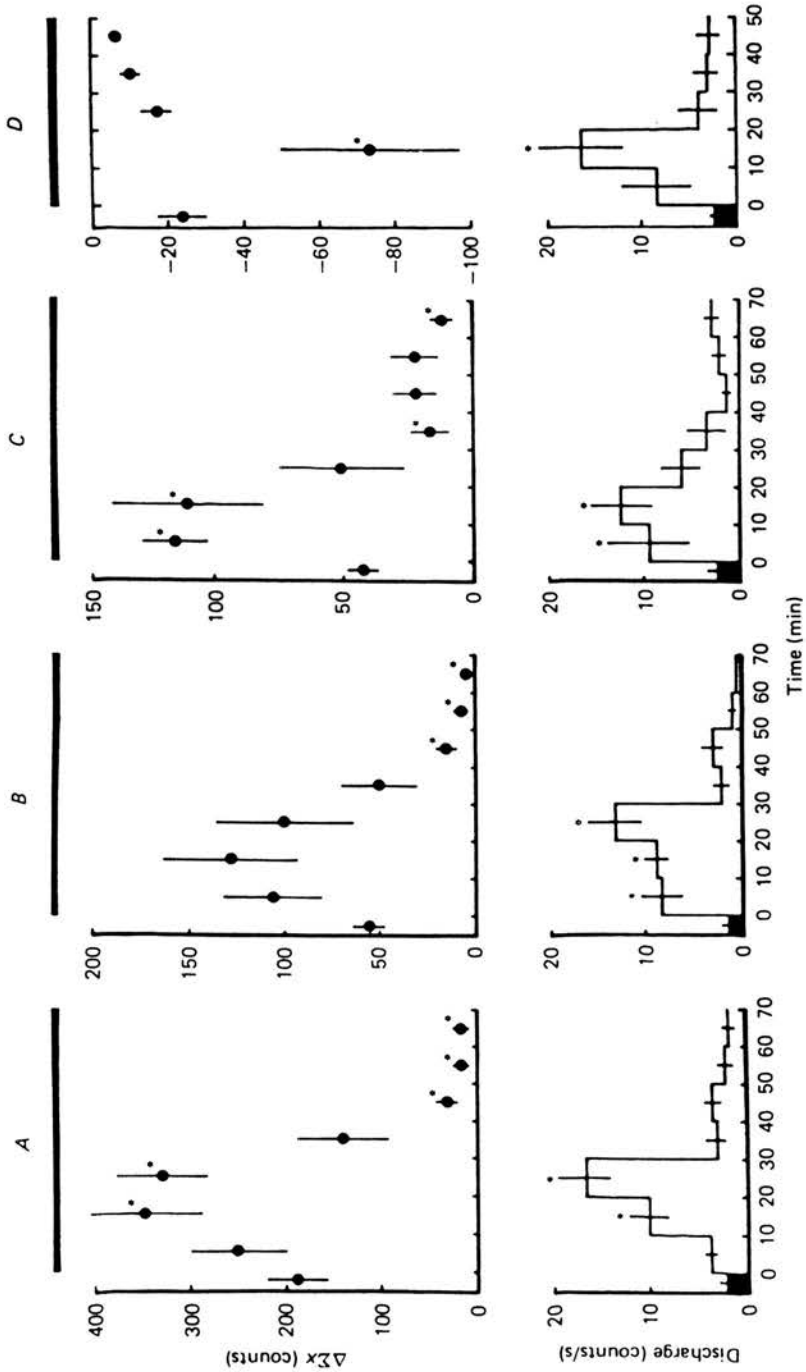

Fig. 6. Effects on chemoreceptor discharge of injecting: *A*, NaCN (5 μg I.C.); *B*, CO_2-equilibrated Locke solution (0·3 ml I.C.); *C*, ACh (50 μg I.C.); *D*, dopamine (5 μg I.C.) before and during ouabain infusion (3 μg/kg I.C.: horizontal bar). In the upper part of the Figure each point is the mean ± S.E. of mean from three to ten experiments, and in the lower part chemoreceptor discharge (counts/s) averaged over 10 min periods during ouabain infusions in the same experiments is shown. The black rectangles represent averaged chemoreceptor discharge observed before starting the ouabain infusion, and the times at which injections of NaCN, CO_2, ACh and dopamine were made are approximate. * $P < 0{\cdot}05$ (*t* test *vs.* control).

10 min periods over which the chemoreceptor discharge was averaged. Since no substantial differences were detected in the responses evoked by these substances in cats with intact ganglioglomerular nerves and those in which they had been cut, responses evoked in both populations were pooled.

Sodium cyanide, CO_2-equilibrated Locke solution and acetylcholine. The chemoexcitatory responses evoked by NaCN, CO_2 and ACh were enhanced during the increase in spontaneous chemoreceptor discharge caused by ouabain and decreased during the

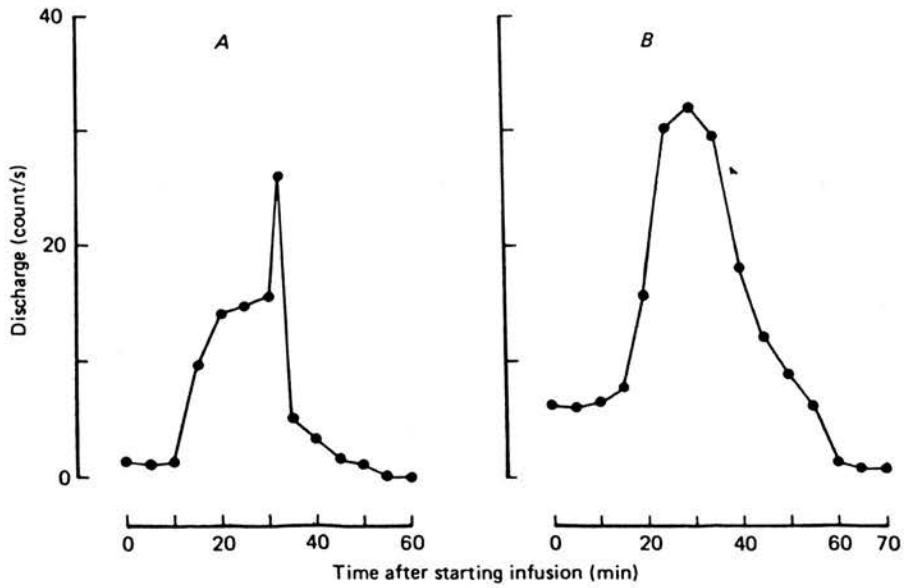

Fig. 7. Effects of ouabain (3 μg/min I.C.) on chemoreceptor discharge in two different cats. *A*, response to ouabain infusion performed after pre-treatment with physostigmine (1 mg/kg I.C.). Recordings obtained from two or three chemoreceptor units in a cat with the ganglioglomerular nerves cut. *B*, response to ouabain after pre-treatment with mecamylamine (5 mg/kg I.C.). Recordings obtained from four to six chemoreceptor units in a cat with intact ganglioglomerular nerves. The infusions of ouabain started at time 0. Dextran was administered intravenously to prevent falls in B.P. occurring following mecamylamine injection.

phase in which discharge returned to pre-infusion (control) levels, or to levels below control. However, the decrease in the ACh responses was not so accentuated as that associated with either NaCN or CO_2 (Fig. 6).

Dopamine. The chemoinhibition evoked by dopamine was enhanced during the increase in spontaneous chemoreceptor discharge induced by the ouabain infusion and was decreased during the subsequent decline in discharge (Fig. 6).

Physostigmine and mecamylamine. Ouabain releases ACh from both central and peripheral nerves (see Gillis & Quest, 1979). In order to determine whether endogenous ACh could be responsible for the increase in chemoreceptor discharge caused by ouabain, three experiments were performed. In one experiment physostigmine (a potent anticholinesterase, effective against both acetylcholinesterase and pseudocholinesterase: e.g. Koelle & Gilman, 1949) was used. Ouabain (3 μg/min I.C.) was administered in this cat pre-treated with atropine (1 mg/kg I.V.) and physostigmine

(1 mg/kg I.C.) and its effect was to induce the usual pattern of changes in chemoreceptor discharges (Fig. 7).

Mecamylamine is a potent ganglion-blocking drug (e.g. Stone, Torchiana, Navarro & Beyer, 1956) which can reduce or abolish the increase in chemoreceptor activity evoked by ACh in the cat carotid body *in vivo* (Sampson, 1971; Nishi & Eyzaguirre, 1971; McQueen, 1977). In two experiments the effects of mecamylamine on the chemoreceptor responses to ouabain were studied. An appropriate volume of dextran solution (25% dextran, 5% glucose in distilled water) was infused intravenously to maintain B.P. at the control level, thereby preventing the severe hypotension which could have increased discharge. Mecamylamine (5 mg/kg I.C.) antagonized the chemoexcitatory action of ACh (25–250 μg I.C.), without affecting the general pattern of the response to ouabain (Fig. 7).

DISCUSSION

The results obtained show that ouabain alters the activity of carotid body chemoreceptors. Its effects can be divided into two components: an initial increase in chemosensory discharge which is followed by a return to, or below, the pre-injection level. Responses evoked by NaCN, CO_2-equilibrated Locke solution, ACh and dopamine were potentiated during ouabain-evoked chemoexcitation, but reduced or abolished during the later post-excitation phase. Thus, ouabain initially sensitizes the chemoreceptors and then desensitizes them.

Several authors have reported effects of ouabain on arterial chemoreceptors (see Introduction) and evidence has been presented that other cardiac glycosides can affect chemoreceptor activity. Schmitt, Güth & Müller-Limmroth (1958) found that digitalis increased carotid chemoreceptor discharge in cats, and more indirect evidence also suggested that cardiac glycosides might activate the chemoreceptors. For example, Heymans, Bouckaert & Régniers (1932) and Zipf & Ehrlicher (1951) concluded that digitalis activates sensory receptors in the carotid sinus and the bradycardia produced by strospeside in cats was considered to result from activation of carotid chemoreceptors (Abiko, 1963). Intracarotid injection of acetylstrophanthidin (a synthetic derivative of strophanthin; ouabain is strophanthin-G) in decerebrate cats caused a smaller increase in activity recorded from the sinus nerve after destroying the carotid bodies with acetic acid (Quest & Gillis, 1971), implying that part of the response to the glycoside resulted from increased chemoreceptor activity. Another glycoside, scillarin A, enhanced the reflex increase in respiration evoked by ACh and nicotine (Carpi, Konzett & Cerletti, 1957), and potentiation of the respiratory stimulant effects of NaCN and ACh by intravenously-administered digoxin has also been observed (Viana, 1973); respiratory stimulation was abolished by surgically removing both carotid bodies from dogs (Viana, 1974). However, the relative contribution made by chemoreceptors, as opposed to other sensory receptors, to these glycoside-induced effects cannot be ascertained from the available evidence.

The present experiments with ouabain have confirmed that cardiac glycosides can increase chemosensory discharge, but in addition we have found that ouabain eventually depresses chemosensitivity. Joels & Neil (1968) also reported that in cats ouabain decreases the chemoexcitation caused by nitrogenated solutions but, in

contrast to our findings, this occurred without initially enhancing the response. Direct comparison of their results with ours is not feasible because the experimental conditions were different and the duration of the ouabain infusions in their experiments is not given.

There are various mechanisms whereby ouabain could affect chemosensory activity. For example, it might be argued that a reduction in blood flow through the carotid body would increase chemoreceptor discharge, and ouabain is known to have a direct effect on vascular beds (e.g. Ribeiro, 1976), can increase vascular resistance on intracarotid infusion (Treat *et al.* 1971) and did increase the systemic B.P. in our experiments. However, although we cannot entirely preclude such a vascular mechanism, we consider it unlikely because moderate alterations in carotid blood flow do not cause sustained changes in chemoreceptor discharge (Biscoe, Bradley & Purves, 1970) and, in any event, chemoexcitation induced by ouabain was still manifest after stopping the infusion, even though any vascular effects of ouabain would have terminated (Treat *et al.* 1971).

It has been claimed that ouabain directly excites the carotid baroreceptors (Quest & Gillis, 1974), and our results could be explained in terms of Biscoe's hypothesis (Biscoe, 1971) that the sensory nerve ending behaves as a chemoreceptor, with ouabain directly affecting the nerve endings. The hypothesis was originally proposed on the grounds that the membrane potential of all nerve cells is determined by ionic concentration gradients which are controlled by the sodium pump. A fall in oxygen tension would slow the pump, allowing a loss of potassium which in turn depolarizes the nerve endings and initiates action potentials in the afferent fibre; it has been shown that raising the extracellular potassium concentration increases chemoreceptor discharge (Jarisch, Landgren, Neil & Zotterman, 1952; Acker, 1978). The sodium pump is inhibited by ouabain (e.g. Repke & Portius, 1963), and it has been postulated that there are two phases in the glycoside's action. Baker & Willis (1972) refer to a primary phase that is a consequence of Na^+–K^+-ATPase inhibition and is concentration-dependent but does not follow simple first-order kinetics. This is followed by a secondary phase resulting from the uptake of ouabain by nerve endings and its subsequent interaction with the movement of calcium ions inside and outside the cells. As far as the present results are concerned, the sensitizing component of ouabain's effect on carotid chemoreceptors would represent inhibition of the sodium/potassium pump, and the later desensitization of these receptors could be related to paralysis of the pump. Considerable latencies occurred between the start of ouabain infusions and the manifestation of significant excitatory effects, and these latencies tended to decrease when infusions were repeated. This might indicate the presence of two components in the mechanism leading to chemoexcitation: the first associated with inhibition of sodium pumping sites located on the cell membrane, the second unsaturable and associated with movement of ouabain in the cell interior (e.g. Baker & Willis, 1972). An early slow component followed by a later exponential component has been demonstrated for ouabain's actions at the frog neuromuscular junction (Brănisteanu, Proca & Haulică, 1979).

The eventual desensitization of chemoreceptors by ouabain seemed not to result from chemoreceptor exhaustion (i.e. absence of responsiveness after severe and prolonged activation of the receptors), because sustained hypoxia (10 % O_2/90 % N_2), even when followed by periods of maximal chemoreceptor discharge evoked by

breathing 100 % N_2, did not affect the responsiveness of the chemoreceptors on return to normal conditions. At the neuromuscular junction 2,4-dinitrophenol (Beani, Bianchi & Ledda, 1966) or a N_2 atmosphere (Hubbard & Løyning, 1966) increase the frequency of miniature end-plate potentials in a manner similar to that of ouabain. The same pattern is generally observed with procedures that interfere with regeneration of ATP (e.g. Alnaes & Rahamimoff, 1975). Although in the present study ouabain appeared to mimic the hypoxic response to some extent, striking differences were noted. First, the response of the chemoreceptors to hypoxia could be maintained throughout the period of stimulation, whereas during ouabain infusion chemoreceptor discharge decreased shortly after peak discharge was reached. Secondly, on return to normal conditions after a lengthy period of hypoxia, chemoreceptor discharge returned to control levels and responses evoked by NaCN, CO_2, ACh and dopamine were not altered substantially, whereas after a ouabain infusion lasting about the same time as the period of hypoxia, responses to these substances were markedly reduced or abolished. Interestingly, spontaneous discharge was generally not appreciably different from the pre-infusion control level. A reason for the differences between ouabain and hypoxia on the carotid chemoreceptors may be that ouabain acts specifically on the Na^+–K^+-ATPase, which behaves as a ouabain receptor, whilst hypoxia acts indirectly by interfering with the synthesis of ATP, as occurs at the neuromuscular junction (Birks & Cohen, 1968).

The carotid body contains several putative transmitters including ACh and noradrenaline (e.g. Biscoe, 1971), and it is well documented that ouabain can release ACh and noradrenaline, both *in vitro* (e.g. Gillis & Quest, 1979) and *in vivo* (Ribeiro & Peres-Gomes, 1977). It also releases adenosine (Shimizu, Creveling & Daly, 1970; Daval, Barberis & Gayet, 1980; Hollins & Stone, 1980; Hollins, Stone & Lloyd, 1980), a substance which can stimulate the chemoreceptors (McQueen & Ribeiro, 1981). Thus, the possibility exists that ouabain affects the chemoreceptors indirectly by modifying the release of one or more substances which may function as transmitters or modulators within the carotid body complex, and is an alternative to mechanisms involving direct actions of the glycoside on sensory endings. The present results do not enable us to establish the contribution made by these various mechanisms to the effects of ouabain. However, ACh appears not to be particularly important since no substantial change in the pattern of chemoreceptor response to ouabain was observed in animals treated with either mecamylamine or physostigmine in doses which would be expected to inhibit or potentiate any endogenous ACh released by ouabain – assuming that these drugs are capable of affecting the actions of endogenously released ACh.

Since ouabain inhibits Na^+–K^+-ATPase, and it is likely that the components of the carotid body contain this enzyme, the response of the chemoreceptors to ouabain may be the net effect of the drug on all the elements of the receptor complex (i.e. type I cells, type II cells, non-myelinated and myelinated sensory nerve endings, motor (efferent) nerve endings). Intracellular recordings have shown that some cells are depolarized by ouabain (see Introduction), but it remains to be established whether this effect is associated with release of particular substances. In order to determine how ouabain acts it will be necessary to correlate changes in chemoreceptor activity induced by the glycoside with biochemical and biophysical changes within individual elements of the carotid body.

Sympathetic nerves to the carotid body appear to be affected by ouabain, since on intracarotid infusion peak discharge occurs following a significantly lower dose of ouabain in intact carotid bodies with intact sympathetic nerves than in those in which the nerves were cut. This may reflect an increase in sympathetic activity, induced centrally or by modification of transmitter release at the ganglia, which increases chemosensory activity. A correlation between carotid body chemoreceptor activation by cardiac glycosides and their cardiotoxic effects has been suggested (e.g. Gillis & Quest, 1979), partly on the grounds that the maximal increase in respiration occurred when the dose reached a level near that required to induce cardiac dysrhythmias (Viana, 1973, 1974). Our animals were artificially ventilated, but the maximal increase in chemosensory activity occurred with doses of ouabain (I.V.) similar to those causing dysrhythmias, which is compatible with Viana's observations. It seems probable that reflexes arising from sensitization of the carotid body chemoreceptors induced by cardiac glycosides acting directly and indirectly will contribute to the cardiovascular changes (e.g. hypertension, bradycardia, dysrhythmia) associated with clinical use of these drugs. This effect may occur at lower doses of glycoside in patients with an elevated level of chemoreceptor activity, which would be expected in hypoxic individuals such as those with certain forms of chronic obstructive pulmonary disease.

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On the specificity and type of receptor involved in carotid body chemoreceptor activation by adenosine in the cat

D.S. McQueen & J.A. Ribeiro[1]

Department of Pharmacology, University of Edinburgh Medical School, 1, George Square, Edinburgh EH8 9JZ

1 Experiments were performed on cats anaesthetized with pentobarbitone in which carotid chemoreceptor activity was recorded from the peripheral end of a sectioned carotid sinus nerve.

2 Intracarotid injections of adenosine 5'-triphosphate (ATP) (1–100 μg i.c.) caused a dose-related increase in chemosensory discharge which was delayed in onset.

3 The adenosine uptake inhibitor dipyridamole potentiated the chemoexcitatory effects of injected adenosine and ATP.

4 The stable ATP analogue α-β-methylene ATP (10–100 μg i.c.) depressed chemoreceptor discharge, which suggests the presence of a P_2-purinoceptor in the carotid body, and provides evidence that the chemoexcitatory effect of ATP results from its hydrolysis to adenosine 5'-phosphate (AMP)/adenosine.

5 Adenine, inosine, guanosine, cytidine and uridine had no appreciable effect on chemoreceptor discharge.

6 The adenosine R-site agonists 2'-chloroadenosine and N^6-methyladenosine had chemoexcitatory effects which were similar to those of adenosine, whereas the P-site agonist 2'-deoxyadenosine had no appreciable effect on discharge.

7 We conclude that the adenosine receptor in the cat carotid body has some of the characteristics of an R-site receptor according to the classification of Londos & Wolff (1977).

Introduction

Adenosine excites the carotid body chemoreceptors (McQueen & Ribeiro, 1981) and so too does adenosine triphosphate (ATP) (Jarisch, Landgren, Neil & Zotterman, 1952; Dontas, 1955; Anichkov & Belen'kii, 1963; Ribeiro & McQueen, 1983) being approximately equipotent in its chemoexcitatory actions with adenosine, the parent nucleoside (Ribeiro & McQueen, 1983). This raises the question of whether it is ATP itself, or the metabolite adenosine, which excites the carotid body chemoreceptors and we decided to investigate the matter by studying the effects on the chemoreceptors of stable analogues of ATP, such as α-β-methylene ATP, which are not metabolized to adenosine.

We also undertook the characterization of the adenosine receptors which are responsible for the chemoexcitation. Two types of receptive site for adenosine have been postulated by Londos & Wolff (1977); one at which agonist activity is favoured by an unsubstituted ribose moiety (R-type), the other at which selective activation is associated with an unsubstituted purine ring (P-type). The effects of various adenosine analogues which are regarded as being selective agonists at either the P- or R-sites were studied on the chemoreceptors, as were substances such as adenine, purine nucleosides and pyrimidine nucleosides in order to test the specificity of the receptors for adenosine.

Methods

Experiments were performed on 23 cats weighing between 2.8 and 4.4 kg, median weight 3.2 kg. They

[1] Present address and correspondence: Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 – Oeiras, Portugal.

were anaesthetized with pentobarbitone sodium (42 mg kg^{-1} i.p.), supplemented as required during the experiments, artificially ventilated with air and paralysed with gallamine (3 mg kg^{-1} i.v.). Full details for most of the experimental procedures have been given previously (McQueen, 1977) and only a brief description follows.

The lingual and superior thyroid arteries ipsilateral to the sinus nerve from which recordings were obtained were both cannulated, the catheter tips being positioned in the common carotid artery. Blood pressure was recorded from a femoral artery. Electrical activity of chemoreceptors (1–6 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, stored on FM tape, passed through a pulse height (window) discriminator and quantified with the aid of a microcomputer (Commodore 3032). In the majority of experiments the ganglioglomerular (sympathetic) nerves were cut.

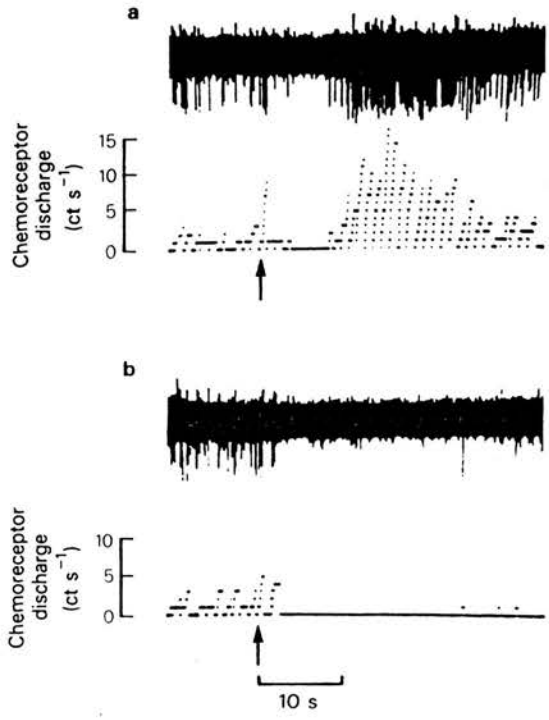

Figure 1 Recording of chemoreceptor discharge illustrating (a) the delayed excitation evoked by an injection (arrow) of ATP (100 μg i.c.) which contrasts with (b), the depression of discharge caused by injecting the same dose of α-β-methylene ATP. The lower part of each panel is the output from a pulse-height discriminator set at a fixed level to count the action potentials occurring during 1 s intervals, each step representing a single spike. The injections lasted 2 s and caused an immediate transient increase in discharge, which was also obtained when the drug vehicle was injected.

Drugs were dissolved in either modified Locke solution (McQueen, 1977) or 0.9% w/v aqueous sodium chloride solution, except for dipyridamole (500 μg ml^{-1}) which was initially dissolved in 25% alcohol:75% polyethyleneglycol. Drug injections were made in a volume of 0.1 ml into the lingual catheter (except for adenosine (100 μg and above) and adenosine analogues (100 μg) which were in 0.2 ml) and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2 : 95% air in a water bath at 37°C; they were made over a 2 s period. Drug infusions were made into the common carotid artery via the thyroid catheter at a rate of 0.1 ml min^{-1} using a Unita pump (Braun) and lasted for 5–10 min; the catheter dead space was 0.2 ml.

Statistical analysis

The significance of differences between means was calculated using Student's paired or unpaired *t* test; *P* values of 0.05 or less were taken as being statistically significant.

Drugs

The drugs used were: sodium pentobarbitone, gallamine triethiodide (May & Baker), adenosine, adenosine triphosphate (ATP), α-β-methylene ATP, β-γ-methylene ATP, 2′-deoxyadenosine, 3′-deoxyadenosine, N^6-methyladenosine, 2′-chloroadenosine, adenine, inosine, guanosine, cytidine, uridine (Sigma), dipyridamole (Boheringer Ingelheim).

Results

Effects of ATP and ATP analogues

Injections of ATP (1–100 μg intracarotid (i.c.)) caused a dose-related increase in chemosensory discharge (see Figures 1, 2 and 3) after a delay of 2–8 s. In view of the delay to onset of the effect, we performed experiments to investigate the possibility that chemoexcitation resulted from actions of a metabolite of ATP rather than the parent compound.

Accordingly, experiments were performed in which the effects of ATP analogues were studied. The actions of α-β-methylene ATP, a compound which cannot be metabolized to adenosine, were compared with ATP. The results obtained are illustrated in Figures 1, 2 and 3 which show that, in contrast to the excitatory effect of ATP, injection of α-β-methylene ATP caused a dose-related decrease in chemosensory discharge that was associated with an increase in arterial blood pressure; ATP had little or no effect on blood pressure. Another ATP

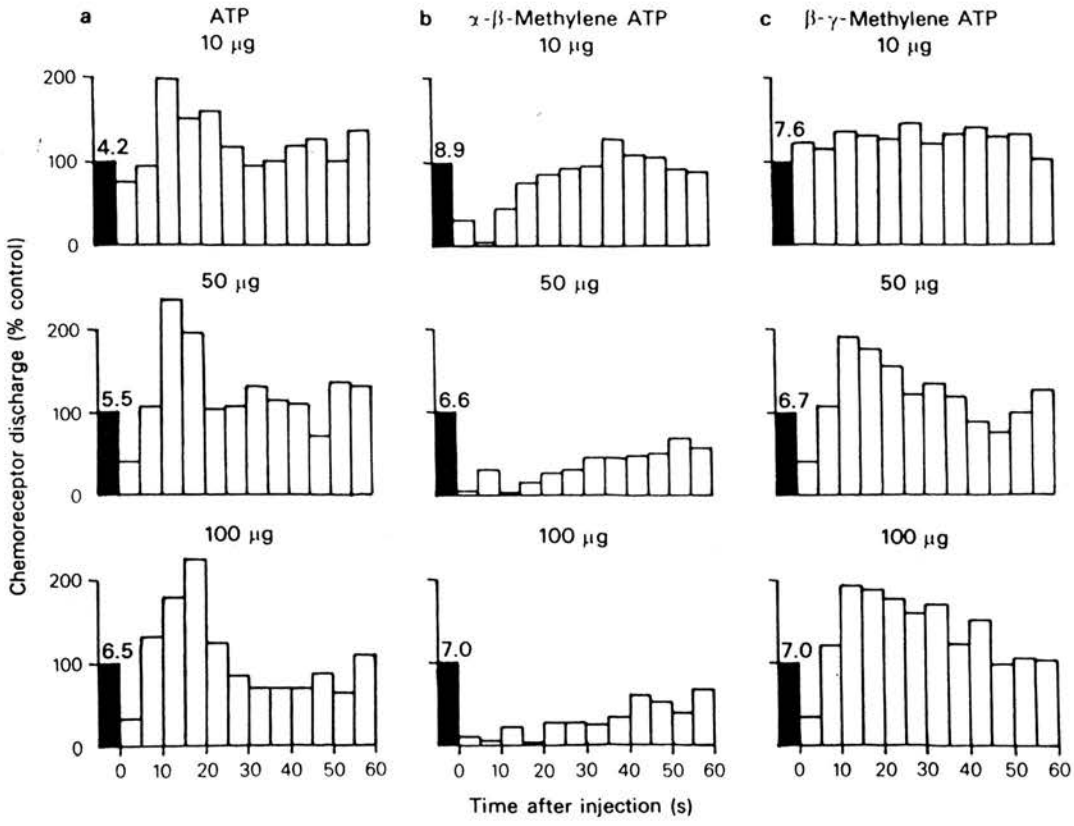

Figure 2 Quantitative comparison of the effects on spontaneous chemoreceptor discharge after injecting 10, 50 and 100 μg of (a) ATP, (b) α-β-methylene ATP and (c) β-γ-methylene ATP into the carotid artery in the same experiment. Discharge was averaged over 5 s intervals and expressed as a percentage of the pre-injection frequency (frequency shown in ct s^{-1} above solid columns which represent 100%).

analogue, β-γ-methylene ATP, which can undergo metabolism to adenosine 5′-phosphate (AMP) and/or adenosine, caused chemoexcitation which was qualitatively similar to the effect of ATP (Figures 2 and 3) and had no appreciable action on blood pressure.

Dipyridamole

The effects of dipyridamole, an adenosine uptake blocker, were studied and it was found that i.v. infusion (50 μg min^{-1}) caused a slight increase in spontaneous chemoreceptor discharge during the period of infusion. The log dose-response curve to adenosine was shifted upwards to the left during the dipyridamole infusion (Figure 4).

Intracarotid infusion of dipyridamole, also at 50 μg min^{-1}, caused a more marked increase in spontaneous discharge. During the i.c. infusion of dipyridamole the increases in discharge evoked by injections of adenosine and of ATP (1 μg) were greatly potentiated (Figure 5).

Specificity for adenosine

A number of nucleosides have actions on neurotransmission (e.g. Phillis, Edstrom, Kostopoulos & Kirkpatrick, 1979), so it was considered of interest to evaluate the effect of some of these substances on the carotid body chemoreceptor activity in order to explore the specificity of the adenosine receptor. Figure 6 summarizes results from 2 experiments in which a metabolite of adenosine, adenine, and the purine nucleosides inosine and guanosine, as well as the pyrimidine nucleosides cytidine and uridine were tested. The effects were compared with those of adenosine itself. As can be seen neither adenine (10–100 μg) nor the nucleosides inosine, guanosine, cytidine or uridine caused any substantial increases in the spontaneous chemoreceptor discharge, whereas adenosine increased discharge.

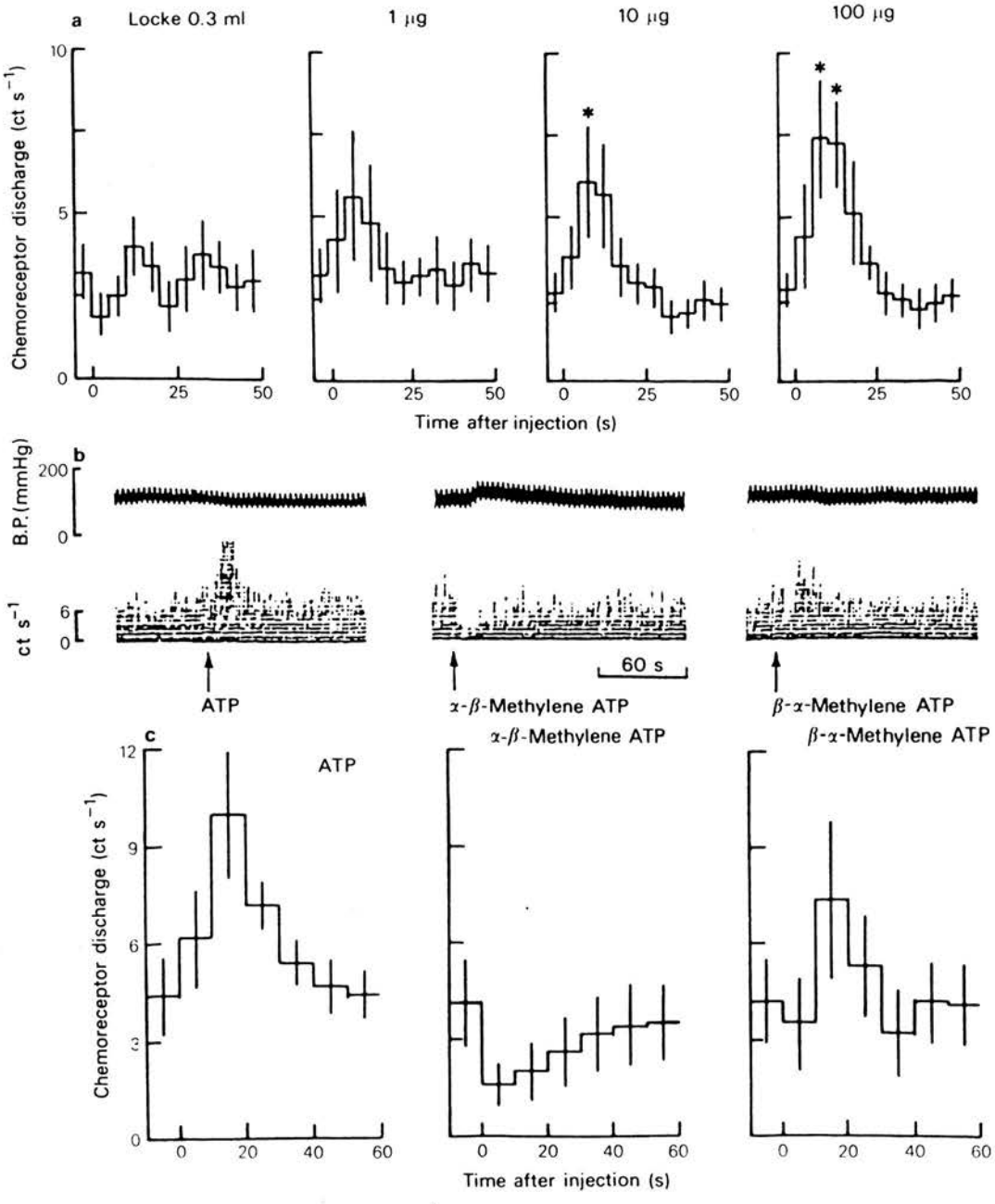

Figure 3 (a) Effects on spontaneous chemoreceptor discharge of injecting Locke solution (0.1 ml + 0.2 ml wash) and ATP 1, 10 and 100 μg i.c. Discharge (ct s^{-1}) was averaged over 5 s periods following the injection and is shown as the mean from 6 experiments ±s.e.mean. * $P < 0.05$ (paired $t$test – comparison with Locke solution). (b) Comparison of the effects of injecting (arrow) 100 μg of non-hydrolysable ATP analogues α-β-methylene ATP and β-γ-methylene ATP with ATP on chemoreceptor discharge and on blood pressure showing the marked difference between α-β-and β-γ-methylene ATP on both parameters. (c) Pooled data from 4 experiments showing quantitatively the effects of these ATP analogues and ATP on chemoreceptor discharge which was averaged over 10 s periods following the injection of 1n00 μg i.c.

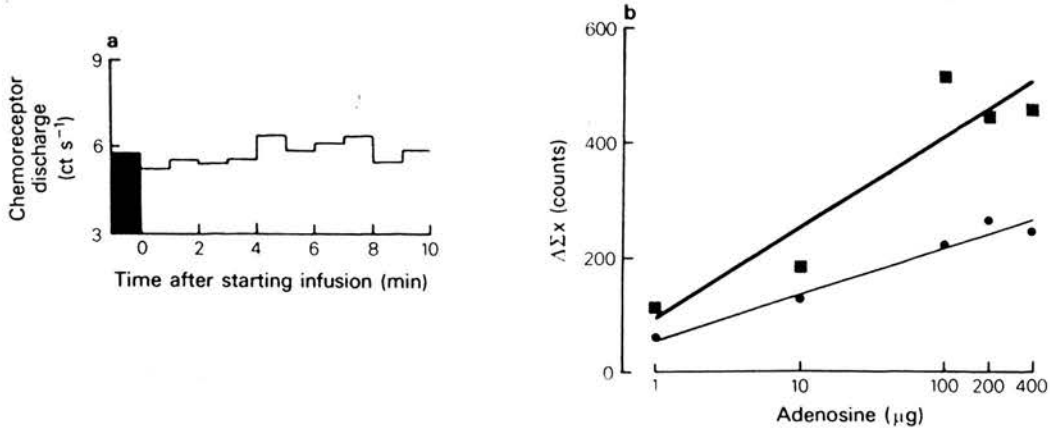

Figure 4 The effect on spontaneous chemoreceptor discharge k(ct/s^{-1}) of infusing dipyridamole (50 μg min^{-1} i.v.) (a). The solid column represents the pre-infusion control discharge frequency. (b) shows log dose-response lines for adenosine injected before (●) and during (■) infusion of dipyridamole (50 μg min^{-1} i.v.). Chemoreceptor responses are expressed as the mean $\Delta\Sigma x$ calculated as the response during the 30 s following injections $\Delta\Sigma x = \Sigma x$(response) $- \Sigma x$(control). Σx(control) = x, (control) × t (response duration in s). x = average discharge in ct s^{-1}. Pre-injection control discharge alues (ct s^{-1}) were: adenosine 1 μg 2.7 before; 2.6 during infusion; 10 μg – 2.0, 2.5; 100 μg – 3.8, 3.3; 200 μg – 3.4, 4.5; 400 μg – 5.8, 4.8. Lines were fitted to the data by the least squares method.

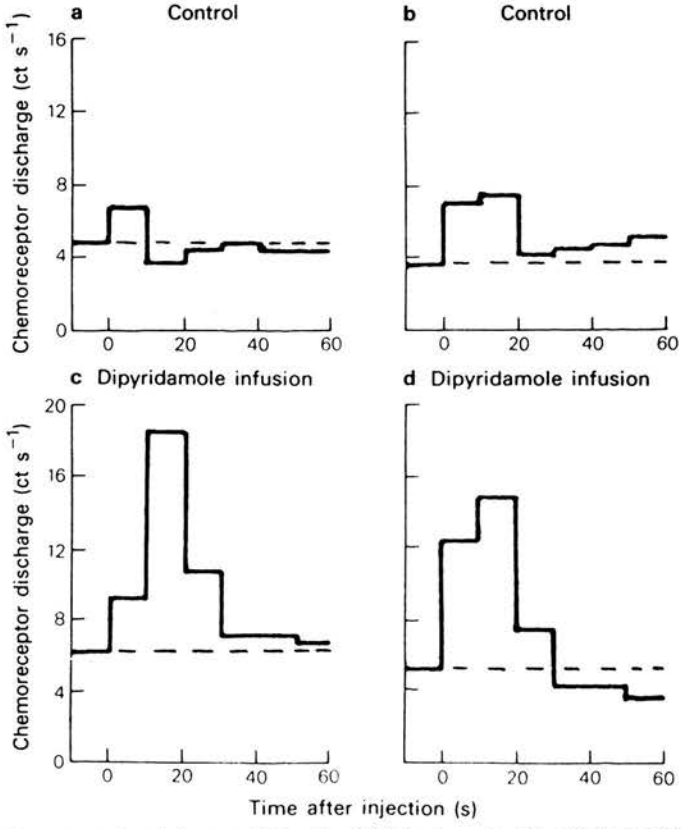

Figure 5 Effects on chemoreceptor discharge of injecting (a),(c) adenosine (1 μg i.c.) and (b),(d) ATP (1 μg i.c.) before and during an infusion of dipyridamole (50 μg min^{-1} i.c.). Discharge (ct s^{-1}) was averaged over 10 s periods. The dashed lines represent the pre-injection averaged discharge.

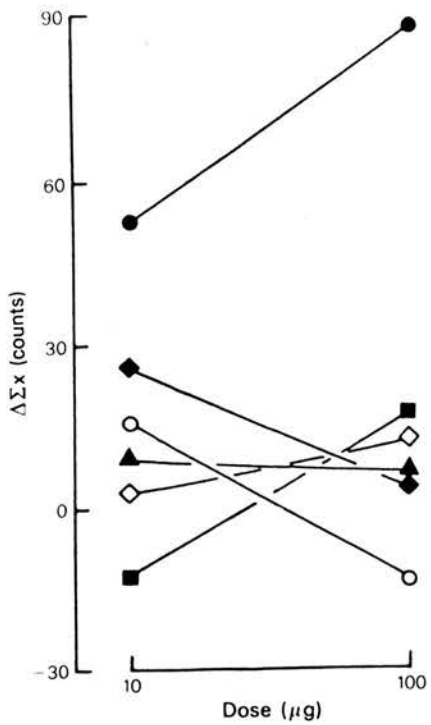

Figure 6 Comparison between the responses of chemoreceptors during the 10–30 s post-injection period to adenosine (●), adenine (○), inosine (▲), guanosine (◆), cytidine (◇) and uridine (■) in 2 cats. $\Delta\Sigma x$ has the same meaning as in Figure 4. Pre-injection control discharge values (ct s^{-1}) for 10 and 100 μg respectively were: adenosine 3.4, 3.0; adenine 2.3, 2.1; inosine, 3.5, 3.2; guanosine, 6.3, 7.8; cytidine 3.1, 2.7; uridine 1.5, 1.2.

Adenosine analogues

The responses of chemoreceptors to adenosine analogues were investigated in 5 experiments. Results obtained from one experiment, in which the effects of the analogues were studied at 2 different doses (10 and 100 μg i.c.), are shown in Figure 7, as are results from 4 experiments in which only the higher dose was used. These demonstrate that N^6-methyladenosine and 2′-chloroadenosine, regarded as R-site agonists, increased spontaneous discharge in a manner similar to that of adenosine. The compound 3′-deoxyadenosine, which can affect both R- and P-sites, but has higher affinity for the former, also increased chemoreceptor discharge. In contrast, 2′-deoxyadenosine, which is an adenosine agonist selective for the P-site had little or no effect on spontaneous discharge.

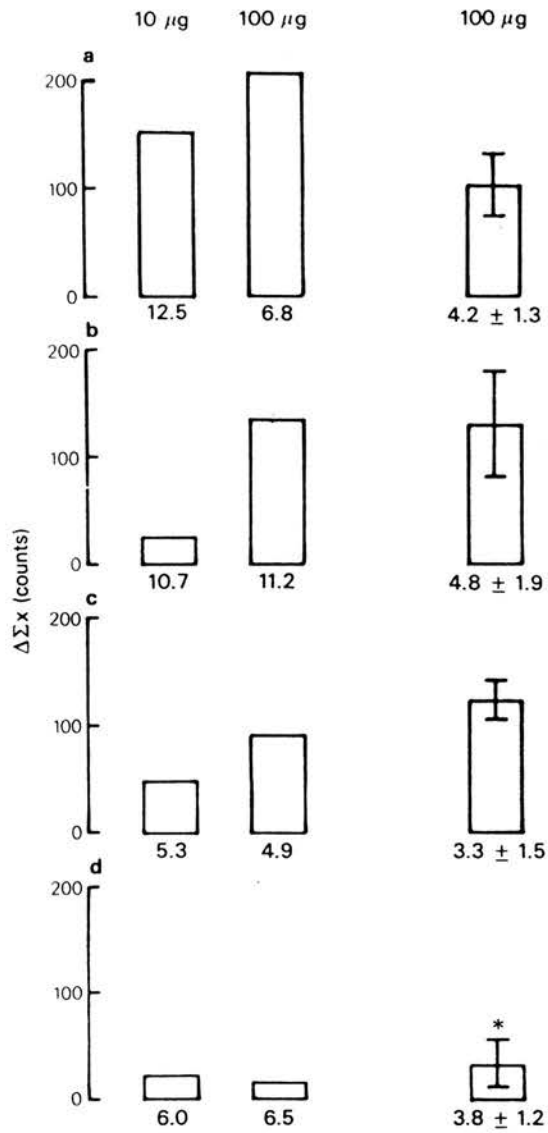

Figure 7 Response of carotid chemoreceptors to injecting 10 μg (first column) and 100 μg (second column) of the adenosine analogues (a) 2′-chloroadenosine, (b) N^6-methyladenosine, (c) 3′-deoxyadenosine and (d) 2′-deoxyadenosine illustrating the dose-dependent nature of the response to the first 3 of the 4 compounds. The third column represents pooled data from 4 further experiments in which the effects of injecting 100 μg of each adenosine analogue were compared with the response to 100 μg of adenosine. Results are expressed as the mean increase in discharge above background activity (i.e. $\Delta\Sigma x$) ± s.e.mean (see Figure 4) during the 10–40 s period following the injection. Background or spontaneous period discharge frequency is shown below each column. Adenosine caused an increase of 170 ± 40 counts, average background discharge 3.0 ± 1.1 ct s^{-1} ($n = 4$). *$P < 0.05$ (comparison with any of the other responses by paired t test).

Discussion

The present results confirm the presence of an adenosine receptor in the cat carotid body (McQueen & Ribeiro, 1981); activation of this receptor causes an increase in chemosensory discharge. Londos & Wolff (1977) postulated the existence of membrane bound adenosine receptors with an internally located P-site and an externally located R-site. The P-site is activated by 2′-deoxyadenosine and the R-site selectively affected by agonists such as N^6-methyladenosine and 2′-chloroadenosine. The present results are compatible with an R-site adenosine receptor being present in the carotid body since the R-site agonists (N^6-methyladenosine, 2′-chloroadenosine) caused effects very similar to those of adenosine, whereas the P-site agonist (2′-deoxyadenosine) had no appreciable effect on chemosensory discharge. However, in many systems the action of adenosine can be blocked effectively by theophylline, whereas in the chemoreceptors it does not seem to be antagonized (McQueen & Ribeiro, 1981). This could mean that a different type of receptor is involved in the response of the chemoreceptors to adenosine, but it should be noted that some adenosine receptors are relatively insensitive to xanthines (Daly, 1983), and complications may arise from the use of theophylline *in vivo* because of the adenosine uptake-blocking and phosphodiesterase-inhibiting properties of the drug. Neither adenine, the purine nucleosides inosine and guanosine, nor the pyrimidine nucleosides cytidine and uridine had any appreciable effect on chemosensory discharge.

The adenosine receptor appears to be externally located since the adenosine uptake antagonist, dipyridamole, potentiated the chemoexcitation evoked by injected adenosine. This further supports the presence of an R-site adenosine receptor in the carotid body, since it is a characteristic of these sites that they are externally located (Daly, Bruns & Snyder, 1981). However, our results do not allow us to establish whether the adenosine receptor is associated with sensory nerve terminals, glomus type I and/or type II cells, blood vessels, or with all these structural components of the sensory complex. It seems unlikely that the effect of adenosine results from direct activation of the sensory axons because adenosine does not change either the amplitude or duration of the compound action potential in frog-sciatic nerve (Ribeiro & Dominguez, 1978) or of action potentials of unmyelinated axons in the brain (Stone, 1981).

ATP did show effects which were similar to those of the nucleoside adenosine, but the nucleotide differed in that there was a delay to onset of the response. This raised the possibility that this effect of ATP on the chemoreceptors depends on its undergoing hydrolysis to AMP and/or adenosine. Experiments with the stable ATP analogue α-β-methylene ATP showed clearly that in this stablized form ATP does not activate chemoreceptors, and indeed it actually depressed discharge. The analogue β-γ-methylene ATP, which can be metabolized to AMP/adenosine, had effects similar to those of adenosine. Therefore, we can conclude that the chemoexcitatory effects associated with ATP are probably attributable to one of its metabolites, possibly adenosine.

The finding that a stable analogue of ATP causes chemoinhibition, whereas adenosine consistently causes chemoexcitation, could mean that in addition to P_1-purinoceptors (Burnstock, 1978), which include both the adenosine P- and R-sites of Londos & Wolff (1977), P_2-purinoceptors, which are activated by ATP, are also present in the carotid body.

According to Londos & Wolff (1977), activating the adenosine P-site would be expected to inhibit the activity of adenylate cyclase, so causing a decrease in levels of cyclic AMP, whereas activation of the R-site is usually associated with activation of adenylate cyclase and would be expected to alter cyclic AMP levels. Whether or not this applies in the cat carotid body remains to be investigated and would help in the classification of the site. The physiological significance of adenosine receptors in this structure remains to be established.

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On the Neuromuscular Depression and Carotid Chemoreceptor Activation Caused by Adenosine

J. A. Ribeiro and *D. S. McQueen

*Gulbenkian Institute of Science, Oeiras, Portugal; and *Department of Pharmacology, University of Edinburgh, Edinburgh, United Kingdom*

This chapter consists of two main sections. In the first, evidence for the action of adenosine and related nucleotides at the neuromuscular junction is reviewed with respect to the following: (a) the type of effect adenosine and related nucleotides have at the neuromuscular junction, (b) their relative potencies, (c) the specificity of their action, (d) the component of the neuromuscular junction at which adenosine acts, (e) the type of purine receptors involved, and (f) some factors that can interfere with the magnitude of the effect. The physiologic and pathophysiologic roles for these substances remain in the domain of speculation. Similar questions as they apply to an *in vivo* investigation of the effects of adenosine on cat carotid body chemoreceptors are considered in the second section. The complexity of the carotid body, particularly the presence of several putative neurotransmitters and the possible existence of two kinds of synapses (motor and sensory), has prevented us from obtaining adequate answers to some of these questions. Nonetheless, it is interesting to note that adenosine, a regulator of oxygen supply to many tissues (see, e.g., ref. 2) affects the carotid body chemoreceptors, which are sensitive to small modifications in the P_aO_2 of blood.

There are, in principle, two distinct actions of adenosine and related nucleotides at synapses. One is the postsynaptic action; adenosine triphosphate (ATP) can behave as a neurotransmitter and/or cotransmitter. The role of ATP as a neurotransmitter has been extensively characterized by Burnstock (3) within the concept of purinergic transmission. For a recent account of the steps that led to the formulation of the purinergic hypothesis, the recently edited monograph *Purinergic Receptors* (4) should be consulted. The function of ATP and/or adenosine as cotransmitters is poorly understood, but basically corresponds to their capacity to sensitize the postsynaptic membrane to the effects of classic neurotransmitters, such as acetylcholine (ACh) or norepinephrine (NE) (see, e.g., ref. 20).

The other action of adenosine at synapses is presynaptic and is manifest by a reduction in transmitter release. This was first detected electrophysiologically by

Ginsborg and Hirst (8,9) in the rat diaphragm and further confirmed within the same laboratory by Ribeiro and Walker (27,28) in the frog sartorius preparation. There is now extensive literature on the presynaptic action of adenosine and adenine nucleotides (see, e.g., ref. 21).

ADENOSINE ON NEUROMUSCULAR TRANSMISSION

In both rat diaphragm and frog sartorius preparations previously depressed either postsynaptically with tubocurarine or presynaptically with high Mg^{2+} and/or low Ca^{2+} concentrations, adenosine reduces the amplitude of the evoked end-plate potentials (EPPs) to about half the control values, which corresponds to a similar reduction in the evoked release of ACh. The evoked release of ACh was measured either by the ratio of the mean amplitude of EPPs to the mean amplitude of miniature EPPs (MEPPs) recorded simultaneously and during the same period or, when the initial quantal content of the EPPs was too low, by determining the natural logarithm of the ratio of the number of impulses to the number of failures. Adenosine also reduces the spontaneous release of ACh as measured by the frequency of MEPPs (9,28). In relation to these effects: (a) adenosine is equipotent with ADP and ATP (28); the effect of ATP might depend on its previous hydrolysis to adenosine, since the nonhydrolyzable ATP analogs α-β-methylene ATP and β-γ-methylene ATP were inactive (32); (b) the effect is specific for adenosine, since substances, such as adenine, the purine nucleosides inosine and guanosine, or the pyrimidine derivatives cytidine and uridine, were ineffective (10); (c) the action of adenosine is devoid of tachyphylaxis (9,28); (d) the effect of adenosine is presynaptic, since the substance does not significantly change the mean amplitude of the MEPPs (9,28); the effect probably is confined to the nerve endings, since adenosine does not change the compound action potential in the frog sciatic nerve (23); (e) the effect of adenosine appears to depend on previous neuromuscular depression; since adenosine is devoid of effect in preparations previously depressed by tetrodotoxin (TTX) (22), this previous neuromuscular depression must take place either postsynaptically via the blockade of ACh receptors (e.g., by tubocurarine) or presynaptically at the nerve endings by using high Mg^{2+} and/or low Ca^{2+} concentrations (24); (f) the effect is antagonized by two substances that have opposite effects on the activity of phosphodiesterases, namely, theophylline and imidazole (24). Both theophylline (29) and imidazole (25) stimulate Ca^{2+} uptake by synaptosomes depolarized by potassium, and the effect of adenosine is accentuated by verapamil (24). This suggests that an interaction with Ca^{2+} probably is responsible for the adenosine-induced neuromuscular depression. Further confirmation comes from the finding that the adenosine reduction of neuromuscular transmission is calcium related (22).

The calcium-related effect of adenosine agrees with its depressant effect on the uptake of Ca^{2+} by synaptosomes stimulated by potassium (26); the depressant effect of adenosine on the evoked release of ACh at the neuromuscular junction results from its decreasing the influx of Ca^{2+} that follows the depolarization of the nerve terminals and triggers the release of transmitter. The reduction in the spontaneous

release of ACh by adenosine and/or ATP might depend on its interaction with the mechanisms that regulate the level of intracellular Ca^{2+} (see, e.g., ref. 23).

Considering the experimental conditions in which the release of ATP from rat phrenic nerve endings has been detected (31), and that the amount of ATP being released together with ACh can be of the same order as that found effective in reducing the output of ACh, it was suggested (21,27) that ATP could be responsible for the Wedenski inhibition.

Physiologic and Pathophysiologic Significance

Some of the points listed above deserve further comment. The possibility of ATP and/or adenosine being responsible for the Wedenski inhibition has been hypothesized (21,27) on the grounds that ATP is released apparently together with ACh from the phrenic nerve endings of curarized preparations after high frequency stimulation (31) and that ATP and/or adenosine in the presence of tubocurarine decrease the evoked release of the transmitter from the motor nerve endings (for more detailed discussions, see ref. 21).

We attempted to test this hypothesis (J. A. Ribeiro, *unpublished observations*) by comparing the Wedenski inhibition obtained before and in the presence of ATP or adenosine applied to a phrenic nerve rat diaphragm preparation set up in similar conditions to those described by Silinsky (31). In some experiments, we have been able to observe a potentiation of the posttetanic inhibition in the presence of adenosine or ATP. Unfortunately, however, these observations are not reliable because it was not possible to obtain consistent controls; i.e., the overall picture of posttetanic inhibition changes independently of the presence of adenosine or ATP. Thus, although we could not prove or disprove the hypothesis, its confirmation might be of interest with respect to a potential role of adenosine on neuronal plasticity.

A point with a pathophysiologic implication emerges from observations in which adenosine appears to be devoid of effect on undepressed preparations (24) and that the neuromuscular depressant effect of adenosine is apparent only if a previous postsynaptic blockade by tubocurarine causes a blockade of at least one-third of the ACh receptors (22). Considering that ATP can be released presynaptically (18,31) and/or postsynaptically (12) and that the neuromuscular blockade induced by tubocurarine has many similarities to that of myasthenia gravis (11), taken together with the report (5) that at the myasthenic end plate, the number of channels opened by a packet of transmitter is decreased about one-third and the number of functional receptors is also reduced in the same proportion, it is possible that an ATP- or adenosine-like substance might be an aggravating factor of a myasthenic-like neuromuscular blockade (22).

ADENOSINE ON CAROTID BODY CHEMORECEPTOR ACTIVITY

In this section, we discuss how exogenously applied adenosine affects carotid body chemoreceptor activity.

Methods

The experiments were performed in cats anesthetized with pentobarbital (42 mg/kg intraperitoneally) supplemented every 1 to 2 hr. The animals were artificially ventilated with air and paralyzed with gallamine (3 mg/kg intravenously). P_aO_2, P_aCO_2, and pH of blood were maintained constant, and end-tidal CO_2, arterial blood pressure (BP), electrocardiogram, and rectal temperature were monitored throughout the experiments. Recordings of action potentials were obtained from the peripheral end of a sectioned carotid sinus nerve using conventional electrophysiologic techniques and quantified (see, e.g., ref. 13). In the majority of the experiments, the ganglioglomerular (sympathetic nerves from the superior cervical ganglion to the carotid body) were cut. Drugs were dissolved in Locke solution and either injected (0.1 ml) or infused into the ipsilateral common carotid artery (IC). The injections were made over a period of 2 sec.

Results and Discussion

Adenosine on Spontaneous Chemoreceptor Activity

As shown in Figs. 1 and 2, adenosine injections (0.1 to 100 μg IC) increased the spontaneous chemoreceptor discharge. This effect was dose dependent in relation to both size and duration of the response, and it was inversely dose related to the delay to onset of the response. The threshold dose for the adenosine excitatory action is between 10 and 100 ng. The effect was significantly different ($p < 0.05$) from the control injection of drug vehicle (Locke solution). Since the injection of Locke solution caused a decrease in chemoreceptor discharge, it is possible that the threshold dose for the adenosine excitatory action is even lower. The effect of adenosine does not appear to be secondary to changes in systemic arterial pressure, since we did not detect any substantial modifications in BP during the period we recorded the adenosine responses, even when we injected the highest dose (100 μg IC) (Fig. 2). No tachyphylaxis to the action of adenosine was observed. Thus when injected at intervals of 5 min over a period of 1 hr, there was no diminution of the effect on spontaneous chemoreceptor discharge.

In another set of experiments, the excitatory action of adenosine was further confirmed by infusing adenosine (50 μg/min IC in 0.1 ml). No substantial changes in BP occurred during the infusions (17).

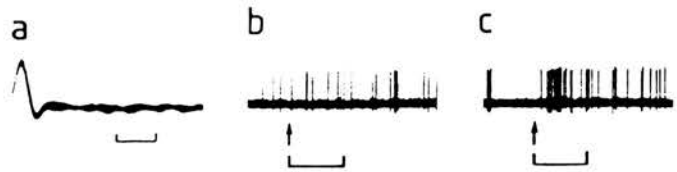

FIG. 1. Effects of IC adenosine on the frequency of spontaneous chemoreceptor discharge. Neurograms taken from one experiment show the increase in the discharge of a single unit caused by injecting *(arrow)* adenosine **(b)** 0.1 μg, and **(c)** 1 μg (10 sec calibration). In **(a)**, the duration of the spike is shown, the trace being of 10 consecutive superimposed spikes (2 msec calibration).

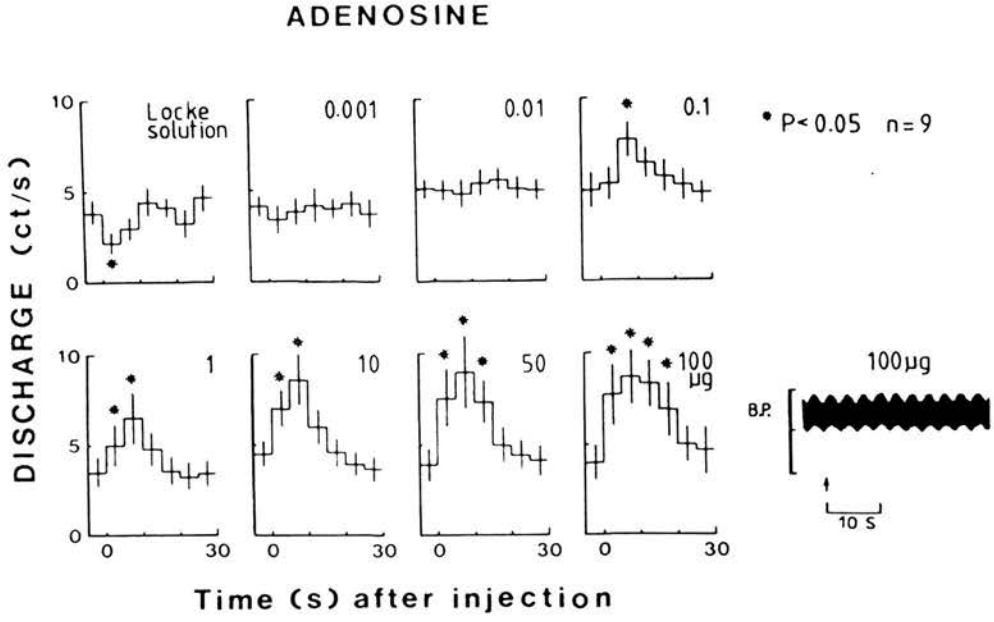

FIG. 2. Effects on spontaneous chemoreceptor discharge (ct/s) of injecting Locke solution (0.3 ml) and adenosine (0.001, 0.01, 0.1, 1, 10, 50, and 100 µg). Discharge was averaged over 5 sec periods following injection. Data obtained from nine cats were pooled and presented as the mean, with vertical bars indicating SEM. The right panel shows an arterial BP trace after injecting *(arrow)* 100 µg adenosine in one experiment. BP calibration, 0 to 100 to 200 mm Hg. *, Compared with preinjection control period.

Dipyridamole

The absence of any relationship between the vasodilator properties of adenosine and its chemoexcitatory action was further confirmed by injecting adenosine (1 to 100 µg IC) in the presence of dipyridamole (J. A. Ribeiro and D. S. McQueen, *unpublished observations*). Dipyridamole, an adenosine uptake blocker, is a potentiator of the vasodilator properties of adenosine (19). Dipyridamole (50 µg/min IC in 0.1 ml) itself increased chemoreceptor activity. In its presence, the effect of the lower doses of adenosine (1 µg IC) was markedly increased without substantial changes in BP, whereas the chemoreceptor responses to the higher doses (100 µg IC) of adenosine were antagonized, and the monophasic excitatory action of adenosine converted into a biphasic action with an early inhibitory component. This effect was clearly associated with a marked fall in BP. The potentiation of the chemoexcitatory action of the lower doses of adenosine during dipyridamole infusion also suggests that the adenosine receptor is externally located.

Comparison Between the Effects of ATP, ADP, AMP, and Adenosine

The effects of ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were investigated in two cats and compared with those of adenosine (see

Fig. 3). All four substances (1 to 100 μg IC) caused a dose-related increase in spontaneous chemoreceptor activity, but the effects of lower doses (1 to 10 μg) of ATP, ADP, and AMP were smaller than those obtained with adenosine. However, if these doses are expressed in moles, ATP (MW, 507) has a similar action to that of adenosine (MW, 267).

Comparing the delay to onset of the adenosine and ATP carotid body chemoreceptor responses, we detected that the responses to ATP usually had a greater delay to onset. This suggests that the effect of ATP might depend on its prior hydrolysis to adenosine.

Adenosine and Evoked Responses

To determine in which of the carotid body structures adenosine acts, we investigated how the substance influenced the chemoreceptor responses evoked by sodium cyanide (NaCN), CO_2-equilibrated Locke solution, ACh, and dopamine.

The general consensus concerning the mammalian carotid body is that it is composed of type I cells and sensory nerve endings enveloped by type II cells; all these components are contained in a rich vascular network. Two kinds of synapses have been proposed; one is efferent or motor, and one is afferent or sensory (for a review, see, e.g., ref. 6). It has been suggested that substances such as NaCN or CO_2 act preferentially on type I cells, and substances such as ACh and dopamine act predominantly on the sensory nerve endings.

Figure 4 illustrates how adenosine influenced the responses evoked by those substances. Responses to NaCN and CO_2-equilibrated Locke solution were slightly and variably reduced after adenosine injections (100 μg IC). This variability was further confirmed by dose-response curves to NaCN performed during the infusion of adenosine. The excitatory responses evoked by ACh and the inhibitory responses evoked by dopamine were both increased after adenosine injections. This effect was confirmed by dose-response curves to both ACh and dopamine obtained during adenosine infusions. Since adenosine sensitized the chemoreceptors to the excitatory

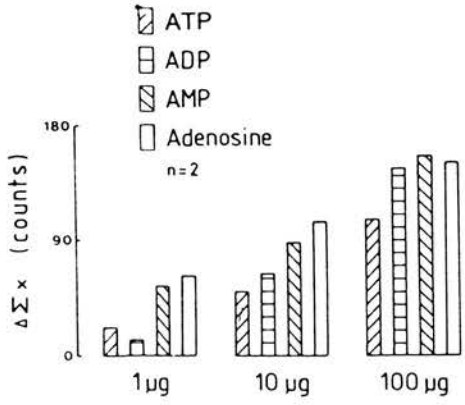

FIG. 3. Comparison between the effects of ATP, ADP, AMP, and adenosine in two cats. Chemoreceptor responses are expressed as the mean $\Delta\Sigma x$ calculated as the response during the 30 sec following injections [$\Delta\Sigma x = \Sigma x$ (response) − Σx (control)]. Σx (control) = $\dot{x}$ (control) × t (response duration in sec). $\dot{x}$ = average discharge in ct/s.

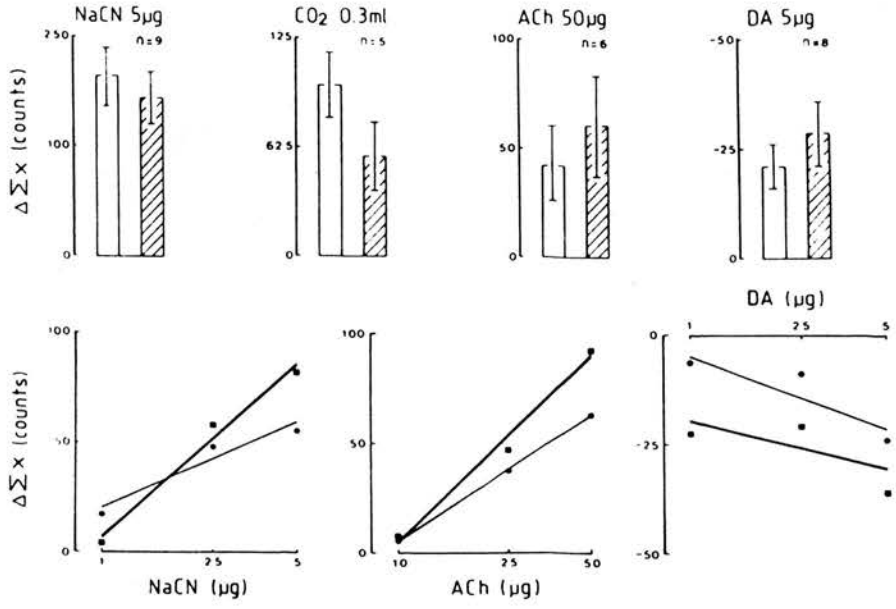

FIG. 4. Effects of adenosine injections (100 µg IC) *(upper panel)* on the chemoreceptor responses evoked by NaCN, CO_2-equilibrated *(open bars)* Locke solution, ACh, and dopamine (DA) before and after *(hatched bars)* adenosine. *Lower panel*, influence of adenosine (50 µg/min IC in 0.1 ml) on the dose-response curves to NaCN, ACh, and DA. *Circles*, before; *squares*, during adenosine infusion. Lines were fitted to the data by the method of least squares.

action of ACh, and in particular to the inhibitory action of dopamine (Fig. 4), adenosine might have a direct action on the sensory nerve endings, which are postsynaptic in relation to type I cells.

Theophylline, Cyclic AMP, and Dibutyryl Cyclic AMP

The possibility that adenosine might act on chemosensory nerve endings also emerges from the finding that theophylline does not antagonize, but instead potentiates, the chemoexcitatory action of adenosine (16,17). Adenosine can activate adenylate cyclase, and theophylline can inhibit phosphodiesterases; i.e., both substances might affect the same ultimate mechanism, namely, cyclic AMP concentration in sensory nerve endings. An adenylate cyclase-cyclic AMP system is apparently located in the sensory endings of the carotid sinus nerve (7); both cyclic AMP and dibutyryl cyclic AMP increase chemoreceptor activity (see Fig. 5).

Another possible explanation for the potentiation by theophylline of the action of adenosine is that theophylline might act by inhibiting adenosine uptake, as has been observed in rat brain synaptosomes (1). The results suggest that the receptors involved in the chemoexcitatory action of adenosine in the cat carotid body are relatively insensitive to blockade by theophylline.

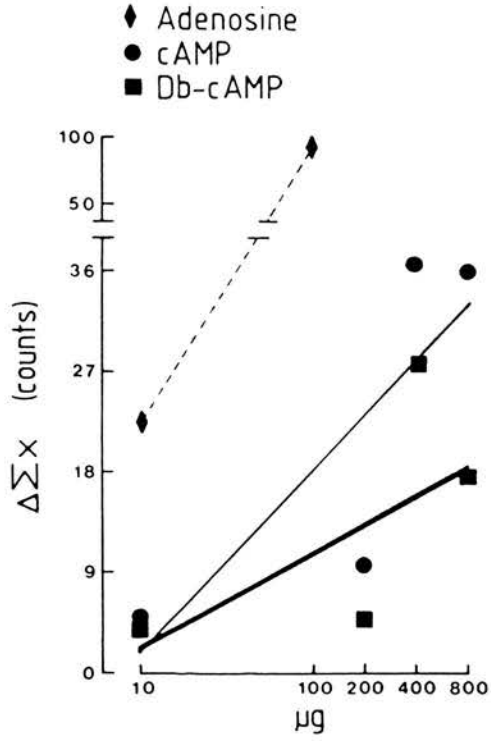

FIG. 5. Effects of cyclic AMP (cAMP) and dibutyryl cyclic AMP (Db-cAMP) on spontaneous chemoreceptor activity and their comparison with adenosine. $\Delta\Sigma x$ has the same meaning as in Fig. 3. Lines were fitted to the data by the method of least squares.

Adenosine is not the Mediator of the Inhibitory Action of Opiates on Carotid Body Chemoreceptors

In contrast to what has been suggested (30,33) for smooth muscle and brain, adenosine does not appear to be the mediator of carotid body chemoinhibitory action of opiates (17). This can be concluded from the following observations: (a) Adenosine does not mimic the action of morphine or enkephalins; these substances cause a rapid and potent inhibitory action on chemoreceptor activity (14,15); (b) theophylline does not cause any substantial change in the inhibitory action of opiates and potentiates the adenosine chemoexcitatory action (J. A. Ribeiro and D. S. McQueen, *unpublished observations*); (c) naloxone, an opiate antagonist, antagonizes the inhibitory action of opiates but does not cause substantial modifications in the excitatory action of adenosine (17).

CONCLUSIONS

The main conclusions that can be drawn from these results are: (a) Adenosine increases spontaneous chemoreceptor activity through an externally located receptor, probably on the sensory nerve endings; (b) since putative inhibitory transmitters are present in the carotid body, and it is well established that adenosine decreases

the release of neurotransmitters in many tissues, we cannot preclude the possibility that this kind of effect may also contribute to the observed adenosine excitatory action. The fact that adenosine is effective in simple systems, such as the neuromuscular junction, as well as in more complex systems, such as the carotid body, emphasizes its importance in neurotransmission, particularly if considered in the context of neurotransmitters, cotransmitters, or neuromodulators.

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SECTION 5

REVIEWS AND MISCELLANEOUS TOPICS

PAPERS 47 - 52

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A method for chronic cannulation of blood vessels in rats

By R. C. Dow* and D. S. McQueen. *Department of Pharmacology and *M.R.C. Brain Metabolism Unit, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ*

The method described here was developed in order to enable drugs to be accurately and rapidly administered intravenously to conscious animals with a minimum of disturbance to the animal, and for this to be repeatable over a period of weeks.

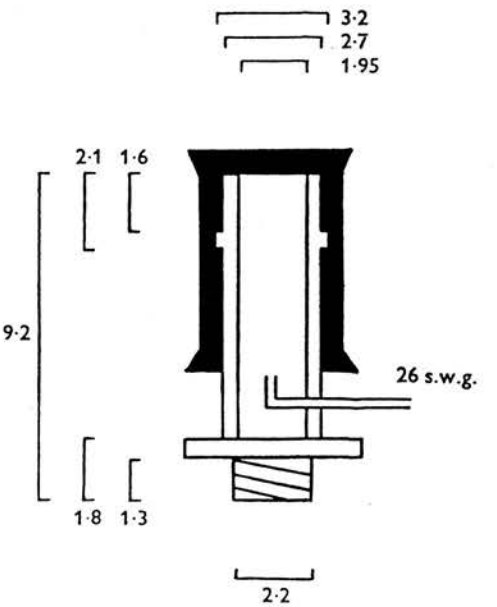

Fig. 1. Cross-section of the skull fitting. Dimensions shown in millimetres. Black area indicates the rubber diaphragm.

The cannulation can be performed on any of the larger blood vessels in the body, but we considered only the external jugular vein. In essence, the method is to cannulate the jugular vein with a nylon catheter and to pass the free end of the catheter under the skin of the neck and fit it to a stainless-steel connector located on the skull. Injections are made through a rubber diaphragm which fits over the stainless steel connector.

Rats of 200–300 g body weight were anaesthetized with halothane in oxygen. Skin incisions were made medial sagittally on the skull and over the jugular vein in the neck-pectoral region. The jugular vein was exposed

[P.T.O.

and cannulated with a nylon catheter (7 cm long with o.d. 0·75 mm), about 1·5 cm of the cannula being in the vein. The free end of the catheter was blocked by a small pin and passed under the skin to the skull incision. A hole was drilled in the skull with a No. 6 dental burr at a point mid-way between the coronal and lambdoidal sutures and 3 mm laterally from the sagittal suture; care should be taken not to penetrate the dura. The hole was then tapped with an 8 BA tap and the stainless-steel fitting (see Fig. 1) screwed into position. The connector was firmly affixed to the skull by applying cold-curing acrylic resin (Simplex), and the catheter attached to the connector.

A hole was punched in the skin directly above the connector to allow the rubber diaphragm to protrude. The wounds were then sprayed with polybactrin antibiotic spray and closed using Michel clips. Surgical time is about 30 min and the wounds heal in 5–7 days. Daily flushing of the cannula with 0·2 ml. heparinized saline (sterile) 100 units/ml. is recommended to ensure patency, and the rubber diaphragm (obtained from a 1 ml. disposable syringe) may need to be changed once every 3 weeks.

Animals have survived for 3 months with patent cannulae; blood samples can be taken using this technique but the cannula should be flushed with sterile heparinized saline immediately after withdrawal.

We should like to thank Dr J. D. Fitzgerald of I.C.I. Ltd. for a gift of halothane.

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THE SPECIFICITY OF SOME AGONISTS AND ANTAGONISTS FOR NICOTINE-SENSITIVE RECEPTORS IN GANGLIA

R.B. BARLOW, FRANCES BOWMAN, R.R. ISON & D.S. McQUEEN
Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ

1 The guinea-pig isolated ileum has been used to estimate the ability of substituted phenylalkylonium salts (related to nicotine) to stimulate or block receptors in ganglia. The effects of hexamethonium were used to indicate which were the most specific ganglion stimulants; these were tested on the blood-pressure of pithed rats and for neuromuscular blocking activity on the rat diaphragm preparation.

2 *m*-Hydroxyphenylpropyltrimethylammonium and 3,4-dihydroxyphenethyltrimethylammonium (coryneine, 'quaternary dopamine') were the most active and specific ganglion stimulants but their usefulness *in vivo* may be limited by their neuromuscular blocking activity. The analogous tertiary compounds are being investigated.

3 The affinities of substances which were blocking agents at ganglionic receptors were measured on the isolated ileum with *m*-hydroxyphenylpropyltrimethylammonium as agonist. The affinities of selected compounds for postganglionic receptors were measured in experiments on the ileum in the presence of hexamethonium and with carbachol as agonist. Some of the compounds were tested for neuromuscular blocking activity on the rat diaphragm.

4 Phenylbutyldiethylamine had ganglion-blocking activity greater than pempidine and little postganglionic blocking or neuromuscular blocking activity. Its triethylammonium analogue had higher ganglion-blocking activity but had appreciable neuromuscular blocking activity.

5 The aromatic ring system is not essential either for activity or affinity and the effects of substituents are not related to their effects on electron distribution. Stimulant activity is enhanced only by hydroxyl or amino groups in suitable positions; it is not improved by the presence of rigid features (double or triple bonds or a cyclopropane ring) in the side chain. Affinity is slightly increased by chloro or bromo groups in suitable positions but the unsubstituted compounds are among those with the highest affinity. Substituents have similar effects on affinity for postganglionic receptors, though for these receptors the compounds mostly have only about one-tenth of their affinity for ganglionic receptors.

Introduction

Barlow, Thompson & Scott (1969) studied the effects of many compounds related to nicotine on the isolated rectus abdominis preparation of the frog. Compounds were found with 50 times the stimulant activity of nicotine; others had considerable blocking activity with values of log affinity constant as high as 5.9.

The present paper describes the properties of some of the most interesting of these compounds at other sites containing nicotine-sensitive receptors, particularly in parasympathetic ganglia. In addition some new compounds have been prepared in attempts to discover substances with high and specific ganglion-stimulant activity and others with high and specific ganglion-blocking activity. These included compounds with substituents not previously examined (fluoro and methyl), a few 3,4-dimethoxy- and 3,4-dihydroxy- compounds, and some unsubstituted compounds with unsaturated or cyclic (and therefore relatively rigid) side-chains. The high nicotine-like activity of 3,4-dihydroxyphenethyltrimethylammonium (the quaternary derivative of dopamine) has already been reported by Cuthbert (1964); this compound is also called coryneine and occurs in the cactus *Stetsonia coryne* (Reti, Arnolt & Luduena, 1935).

The activity and specificity of ganglion-stimulants was tested on the guinea-pig isolated ileum by assessing activity relative to a standard known to be reasonably specific and repeating the assessment in the presence of hexamethonium. Selected compounds were then tested separately

with a range of concentrations of hexamethonium, in order to see how far the dose-ratios obtained were consistent with competition. Compounds with ganglion-stimulant activity were also tested on the blood-pressure of pithed rats.

The most specific ganglion-stimulant was then used as an agonist in order to assess the affinity of antagonists for ganglionic receptors in the guinea-pig ileum. The extent to which the results were consistent with competition gave some indication of the specificity of the block. A rough idea of the neuromuscular blocking activity of many of the compounds was obtained by tests with the rat diaphragm preparation. The affinity constants of some of the compounds for postganglionic receptors in the ileum were also measured.

Methods

Guinea-pig ileum

The guinea-pig ileum was set up in aerated Tyrode solution at 37°C; responses were recorded isotonically with a load of about 0.5 gram. The interval between doses was 5 min, as described by Barlow & Franks (1971), to avoid desensitization. The agonist was in contact with the tissue for 30s and the drug solutions were applied by automated apparatus (Abramson, Barlow, Mustafa & Stephenson, 1969; Edinburgh Staff, 1970).

Agonists In the first group of experiments the activity of agonists was assessed relative to a standard, *p*-aminophenethyltrimethylammonium. Groups of responses were obtained with two concentrations of the standard, then with two concentrations of the test drug, and then again with two concentrations of the standard. The concentrations producing comparable responses were calculated from the average responses and the activity was expressed as the equipotent molar ratio, i.e.

$$\frac{\text{concentration of test}}{\text{concentration of standard}}$$

Mean values of the log ratio were calculated ± the s.e. mean.

In the second group of experiments the comparison with the standard was performed in exactly the same way but the Tyrode solution contained hexamethonium (3×10^{-5}M). From the estimate of the affinity constant of hexamethonium obtained by Barlow & Franks (1971), this should produce a dose-ratio of 8.8 but if the agonists are all acting at the same receptors as those blocked by hexamethonium, this should not affect their relative activities.

Some of the agonists were tested further by measuring the dose-ratios produced by various concentrations of hexamethonium. Responses were obtained with two concentrations of the agonist in the absence of hexamethonium and then with increased concentrations of agonist in the presence of hexamethonium. Usually two or three concentrations of hexamethonium were tested in any one experiment but in the present work the dose-ratio was calculated using only the responses obtained in the absence of hexamethonium at the start of the experiment. In the work of Barlow & Franks (1971) responses were also obtained in the absence of hexamethonium after two concentrations of hexamethonium had been tested but these were quite often found to differ appreciably from those obtained at the start of the experiment; it is probably more satisfactory to test a smaller number of concentrations of hexamethonium in any one experiment and to perform experiments with more pieces of ileum.

Antagonists The antagonism produced by compounds was assessed by their effects on the responses to *m*-hydroxyphenylpropyltrimethylammonium, which seemed to be the most suitable agonist (see results). Each antagonist was usually tested in two concentrations, producing dose-ratios of about 10 and about 20, and the exact dose-ratio and concentration of antagonist were used to calculate the affinity constant. Results were expressed as the mean value of log affinity constant ± the s.e. Some compounds did not act competitively and could be tested only in one concentration because the antagonism was unsurmountable. This could be due to a postganglionic blocking action (i.e. atropine-like activity) and only a rough idea of their effects at ganglionic receptors could be obtained.

Postganglionic receptors The affinity constants of selected compounds for postganglionic receptors in the ileum were measured in experiments with carbachol as agonist, allowed to act for 30s and given once every 90s, and with hexamethonium, 2.76×10^{-4}M, present in the Tyrode solution (Barlow, Scott & Stephenson, 1963; Edinburgh Staff, 1970; Barlow, Franks & Pearson, 1973). The selection was intended to show the effects of chain length and of type of substituent on affinity, for comparison with their effects on affinity for receptors in ganglia. Some compounds were also included for which the results in the experiments on ganglionic receptors (see above) suggested that they should also be acting postganglionically.

Effects on blood-pressure

Pithed male Wistar rats, weighing between 250 and

500 g, were anaesthetized with ether; the trachea was cannulated, and a 14 gauge needle passed through the eye and down the spinal cord. Ventilation was maintained artificially with air from a Palmer pump, at a rate of 1 stroke/s and with a stroke volume of 5 ml. The animal was kept on a heated operating table and the left external jugular vein was cannulated with a nylon catheter (0.63 mm o.d.), which was used for administering drugs. The left carotid artery was cannulated with a nylon catheter (0.75 mm o.d.) which was connected to a Consolidated blood-pressure transducer attached to a Devices M2 hot-stylus recorder. Heparin (200 units) and atropine sulphate (0.4 mg; 1.14 mmol) were administered and the mean blood-pressure was recorded continuously.

Drugs were given by infusion through the vein cannula with a Watson Marlow MRHE pump in a period of 15 seconds. Different doses were given by altering the rate of infusion and the interval between doses was 4 to 5 minutes. The activity of compounds was expressed relative to that of a standard, *p*-aminophenethyltrimethylammonium, by comparing doses producing comparable rises in pressure. The standard was tested twice at three dose-levels during each experiment and it was usually possible to compare two or three compounds with it in any one animal also at three dose-levels tested twice. The rise in blood-pressure observed was biphasic. The second rise was absent after adrenalectomy and the first rise was blocked by bretylium. Both these effects were expressed as the rise in pressure divided by the blood-pressure before the drug was given (which did not vary much during the experiment) and the values were used to calculate log dose-response lines by the method of least squares. Equipotent molar ratios were calculated from the ratios of the doses producing responses which were 20% and 80% of the maximum observed. With the particular compounds tested, these equipotent molar ratios for the two phases in the response were approximately the same so the results could be expressed as a single ratio.

Rat diaphragm

The rat isolated diaphragm preparation was set up as described by Bülbring (1946) but in Krebs solution instead of Tyrode (Edinburgh Staff, 1970). Responses were recorded isometrically with a Devices strain-gauge and recorder. The phrenic nerve was stimulated once every 10s by rectangular pulses of about 0.5 ms duration, at a voltage (usually about 2V) which produced maximal twitch responses. Drugs were added by hand once every 15 min and allowed to act for 5 minutes. In each experiment compounds were tested in doses of 0.02 and 0.04 ml of 10^{-1}M solutions and 0.10 ml of 10^{-2}M; the bath volume was approximately 20 ml. The percentage inhibition of the contractions was calculated and the total ('score') for the three doses was taken. Each compound was tested on two preparations and the average score used as a very rough estimate of relative neuromuscular blocking activity.

Compounds

The new compounds are listed in Table 1. The other compounds tested have been described by Barlow, *et al.* (1969). Pempidine tartrate, included in the tests of ganglion-blocking activity, was obtained from May & Baker; nicotine hydrogen tartrate from BDH Ltd; dimethylphenylpiperazinium iodide (DMPP) from Aldrich.

The synthesis of some other new substituted phenylpropyl compounds used has been described elsewhere (Ison & Hassan, 1973).

Trans-2-phenylcyclopropyltrimethylammonium was prepared from the amine (Burger & Yost, 1948; Smejkal & Farkas, 1963); phenylprop-1-ynyltrimethylammonium was prepared from the dimethylamino compound (Iwai & Hiraoka, 1963), which was also partially hydrogenated using Lindlar catalyst and a drop of quinoline to the *cis* form of 3-dimethylamino-1-phenylprop-1-ene and quaternized. *Trans*-3-dimethylamino-1-phenylprop-1-ene was prepared by dehydration of the alcohol (Ison & Casy, 1971); the assignment of configuration was supported by the intervinylic coupling constants (J= 16 Hz for the *trans*-dimethylamino compound; J= 8 Hz for the *cis*-isomer; both appeared to be isomerically pure).

Difficulties were experienced in the repetition of the synthesis of leptodactyline (*m*-hydroxyphenethyltrimethylammonium). Samples were obtained which had m.p. 251-2°C (cf. 163-4°C) and had low biological activity even though the analysis was acceptable for leptodactyline bromide (found Br^-, 30.94; theory, 30.70%). the n.m.r. spectrum however, indicated that this, and the methoxycompound from which it was derived, were tetrahydro*iso*quinoline derivatives, formed by Pictet-Spengler cyclisation during the methylation of *m*-methoxyphenethylamine. 6-Methoxy-2-methyl-1,2,3,4-tetrahydro*iso*quinoline methiodide had m.p. 170-173°C (found I^-, 39.77, theory, 39.75, cf. *m*-methoxyphenethyltrimethylammonium iodide, m.p. 182.3-182.6°C, theory, I^- 39.51). The theoretical content of bromide for 6-hydroxy-2-methyl-1,2,3,4-tetrahydro*iso*quinoline methobromide is 30.94, identical with that found. *m*-Methoxyphenethyldimethylamine was therefore prepared from the reduction of the dimethylamide (Epstein, Plapinger, Michel, Cable,

Stephani, Hester, Billington & List, 1964) and the methobromide had m.p. 163-4°C.

Results

Ganglion stimulants

The relative activity of the compounds on the guinea-pig ileum is shown in Table 2. Several of them were more active than the standard, *p*-aminophenethyltrimethylammonium, but some of these were even more active in the presence of hexamethonium (with positive values of Δ in Table 2) indicating that they were acting, at least in part, at postganglionic receptors (or ganglionic receptors not blocked by hexamethonium). This was most marked with the β-pyridylmethyl, phenethyl, cyclopentylpropyl, phenylcyclopropyl, phenylpropynyl compounds and leptodactyline. With

Table 1 Melting points and analyses of new compounds.

$R'C_6H_4CH_2\overset{+}{N}Me_3\ X^-$					
R' =		*X*⁻ =	*m.p.*	*Found (X⁻)*	*Theory*
o-Me		Br^-	206-8	32.62	32.72
m-Me		Br^-	218-9	32.58	32.72
p-Me		I^-	213	43.73	43.58
p-F		I^-	248	42.71	42.99
$R''R'C_6H_3CH_2CH_2\overset{+}{N}Me_3\ X^-$					
R'' =	*R'* =				
m-F	H	I^-	232-3	40.85	41.04
p-F	H	I^-	251-4	40.86	41.04
p-MeO	*m*-MeO	I^-	230	36.00	36.14
p-HO	*m*-HO	I^-	207-9*	C, 41.1	40.9
				H, 5.46	5.61
$R''R'C_6H_3CH_2CH_2\overset{+}{N}Et_2H\ X^-$					
p-Br	H	Cl^-	142	12.16	12.12
p-MeO	*m*-MeO	Cl^-	126-7	13.19	12.95
$R''R'C_6H_3(CH_2)_3\overset{+}{N}Me_3\ X^-$					
p-Me	H	I^-	119-120	39.45	39.76
p-MeO	*m*-MeO	I^-	133	34.68	34.75
p-HO	*m*-HO	Br^-	200-201	C, 49.7	49.7
				H, 7.08	6.95
$BrC_6H_4CH{=}CHCH_2\overset{+}{N}Et_2H$	*trans*	Cl	161	11.69	11.65
$C_6H_5C{\equiv}CCH_2NMe_3\ I^-$			226-8	42.01	42.13
$C_6H_5CH{=}CHCH_2\overset{+}{N}Me_3\ I^-$	*cis*		116-7	42.16	41.86
	trans		182-3	42.02	41.86
$C_6H_5\Delta\overset{+}{N}Me_3\ I^-$	*trans*		182-4	42.23	41.86
$C_6H_5(CH_2)_4\overset{+}{N}Et_2H$		Br^-	99-100	27.85	27.90
$C_6H_5(CH_2)_5\overset{+}{N}Et_2H$		Br^-	79	26.62	26.61
$C_5H_9(CH_2)_3\overset{+}{N}Me_3$		I^-	208	42.72	42.70
$C_5H_9(CH_2)_3\overset{+}{N}Et_2H$		Br^-	95-97	30.32	30.22
$C_6H_{11}(CH_2)_3\overset{+}{N}Me_3$		I^-	173	40.56	40.78
$C_6H_{11}(CH_2)_3\overset{+}{N}Et_2H$		Br^-	118-9	28.72	28.72

Melting points were measured with a Mettler FP1 instrument, usually at a rate of heating of 2°C/min, and are uncorrected. Analyses for halide are gravimetric (with samples of 50-250mg) and for C and H are micro- by Drs Weiler and Strauss, Oxford. The quaternary derivative of dopamine, marked with an asterisk, was prepared by Barger & Ewins (1911), who recorded m.p. 205°C and m.p. 232°C for the dimethoxy compound. Details of other compounds studied are given by Barlow, *et al.*, (1969) and Ison & Hassan (1973).

some others hexamethonium reduced activity relative to *p*-aminophenethyltrimethylammonium. In most instances this indicated that the compounds had postganglionic blocking activity and this was investigated in experiments with carbachol as agonist (see below). The variation in the effects of hexamethonium on the actions of the phenylpropyl compound (Table 2) could be ascribed to a lower sensitivity of the receptors in ganglia in the second group of experiments with the consequent need to use higher concentrations which produced detectable postganglionic blocking effects.

The compounds which were considered to be most interesting were tested in the presence of various concentrations of hexamethonium in order to see to what extent the antagonism appeared to

Table 2 Stimulant activity on the isolated guinea-pig ileum.

	Hexamethonium concentration		
	0	*3 x 10⁻⁵ M*	Δ
β-pyridylCH$_2$ $\overset{+}{N}$Me$_3$	$\bar{1}$.25 ± 0.06 (5)	$\bar{2}$.73 ± 0.06 (3)	+0.52
β-pyridylCH$_2$ CH$_2$ $\overset{+}{N}$Me$_3$	$\bar{1}$.52 ± 0.02 (4)	$\bar{1}$.35 ± 0.01 (2)	+0.17
m-HOC$_6$H$_4$(CH$_2$)$_3$NMe$_3$	$\bar{1}$.69 ± 0.02 (5)		
pm-(HO)$_2$C$_6$H$_3$CH$_2$ CH$_2$ $\overset{+}{N}$Me$_3$ (coryneine)	$\bar{1}$.82 ± 0.01 (4)	$\bar{1}$.68 ± 0.02 (3)	+0.14
m-HOC$_6$H$_4$CH$_2$CH$_2$ $\overset{+}{N}$Me$_3$ (leptodactyline)	$\bar{1}$.92 ± 0.02 (5)	$\bar{1}$.07 ± 0.02 (4)	+0.85
p-H$_2$NC$_6$H$_4$CH$_2$ CH$_2$ $\overset{+}{N}$Me$_3$	0.0 (standard)	0.0	
C$_6$H$_5$CH=CHCH$_2$ $\overset{+}{N}$Me$_3$ *trans*	0.07 ± 0.01 (4)	0.47 ± 0.04 (3)	−0.40
Ph(CH$_2$)$_2$ $\overset{+}{N}$Me$_3$	0.20 ± 0.02 (3)	$\bar{1}$.4	+0.8
pm-(HO)$_2$C$_6$H$_3$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$	0.42 ± 0.03 (6)	0.35 ± 0.02 (3)	+0.07
C$_6$H$_5$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$ (1)	0.60 ± 0.04 (5)	0.68 ± 0.01 (3)	−0.08
(2)	0.61 ± 0.01 (3)	0.91 ± 0.1 (2)	−0.3
p-HOC$_6$H$_4$CH$_2$CH$_2$ $\overset{+}{N}$Me$_3$ (hordenine methiodide)	0.79 ± 0.04 (4)		
C$_5$H$_9$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$	0.84 ± 0.02 (3)	0 approx.	+0.8
p-HOC$_6$H$_4$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$	0.86 ± 0.03 (4)		
Phenylcyclopropyl $\overset{+}{N}$Me$_3$ *trans*	0.90 ± 0.06 (3)	0 approx.	+0.8
m-H$_2$NC$_6$H$_4$CH=CHCH$_2$ $\overset{+}{N}$Me$_3$ *trans*	0.91 ± 0.01 (4)		
p-H$_2$NC$_6$H$_4$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$	1.03 ± 0.01 (4)		
C$_6$H$_5$C≡CCH$_2$ $\overset{+}{N}$Me$_3$	1.12 ± 0.08 (3)	0.3 approx.	+0.8
2-methyl-7-hydroxytetrahydroisoquinoline methiodide*	1.60 ± 0.06 (2)		

The numbers show the mean estimate of the logarithm of the equipotent molar ratio relative to *p*-aminophenethyltrimethylammonium ± s.e., with the number of estimates in parentheses. Note that a negative number indicates greater activity because the same responses are produced with smaller concentrations. The column marked Δ shows the effect of hexamethonium: if the compound is as specific as the standard the value should be zero. A positive value suggests postganglionic stimulant (muscarine-like) activity; a negative value suggests postganglionic blocking (atropine-like) activity. The phenylpropyl compound was tested in two separate sets of experiments (1) and (2), and the effects of hexamethonium were found to be variable, though the relative activity in the absence of hexamethonium was the same. The compound marked with an asterisk was found accidentally in the synthesis of leptodactyline (see above) and is only very feebly active.

m-F$_3$CC$_6$H$_4$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$, C$_6$H$_5$(CH$_2$)$_4$ $\overset{+}{N}$Me$_3$ and C$_6$H$_{11}$(CH$_2$)$_3$ $\overset{+}{N}$Me$_3$ appeared to be inactive but the two latter blocked the actions of carbachol. C$_5$H$_9$(CH$_2$)$_3$NMe$_3$ appeared to be a partial agonist at postganglionic receptors with log K about 4.2.

be competitive; the results are shown in Table 3. These agree reasonably with results already obtained for two of the compounds by Barlow & Franks (1971). There are differences, which suggest that the errors may be bigger than the variance indicates, but these could partly be due to differences in experimental technique (discussed in the methods section). With the lower concentrations of hexamethonium there is good agreement between the experimental dose-ratios for most of the compounds and those calculated assuming a value of 2.6×10^5 for the affinity constant of hexamethonium.

The low dose-ratios obtained with β-pyridylethyltrimethylammonium show that it acts to a large extent at receptors not blocked by hexamethonium and the high dose-ratios obtained with phenylpropyltrimethylammonium indicate that it has appreciable postganglionic blocking (atropine-like) activity, though this is not as much as that of dimethylphenylpiperazinium (DMPP), for which Barlow & Franks (1971) obtained dose-ratios of 12 with only 2×10^{-5} M hexamethonium.

From the results obtained with the highest concentration of hexamethonium, 1.6×10^{-4} M, it seemed that *m*-hydroxyphenylpropyltrimethylammonium was the most specific in that the results were closest to those consistent with competition. It was therefore chosen as the agonist for testing ganglion-blocking activity with the preparation. The results obtained with *p*-aminophenethyltrimethylammonium were not as consistent with competition as those previously obtained (Barlow & Franks, 1971), but these did not include experiments with 1.6×10^{-4} M hexamethonium. The results confirm the high nicotine-like activity of the quaternary derivative of dopamine (coryneine), reported by Cuthbert (1964), but the compound is more difficult to make than the *p*-aminophenethyl- and *m*-hydroxyphenylpropyl- compounds and slowly oxidizes in solution, turning pink, so it was considered less suitable for extensive use as a ganglion-stimulant. It was, however, slightly more active than these compounds on the rat blood-pressure (Table 4).

Most of the ganglion-stimulants were also potent blocking agents on the rat diaphragm (Table 5) so their potential usefulness *in vivo* might be limited by their neuromuscular blocking properties. It was not possible to assess this from the experiments on pithed rats because these were artificially ventilated, but the lowest concentration

Table 3 Dose-ratios produced by hexamethonium with different agonists on the guinea-pig ileum.

	Hexamethonium concentration (x 10^{-5} M)				*Agonist concentration*
	2	*4*	*8*	*16*	
Calculated dose-ratio	6.2	11.4	21.8	42.5	
p-$H_2NC_6H_4CH_2CH_2\overset{+}{N}Me_3$	7.2*	14.7*	21.0*		
	4.7	8.1	14.4	19.0	4×10^{-6} M
	±0.5	±0.3	±1.0	±2.9	
	(2)	(2)	(7)	(2)	
m-$HOC_6H_4(CH_2)_3\overset{+}{N}Me_3$	6.4*	10.3*	15.9*		$2.\times 10^{-6}$ M
			17.6	52.0	
			±2.3	±1.3	
			(3)	(2)	
pm-$(HO)_2C_6H_3CH_2CH_2\overset{+}{N}Me_3$		12.4	20.4	69.4	3×10^{-6} M
		±1.6	±2.6		
		(2)	(3)	(1)	
pm-$(HO)_2C_6H_3(CH_2)_3\overset{+}{N}Me_3$		11.6	18.0		7×10^{-6} M
		±0.4	±1.5		
		(3)	(3)		
$C_6H_5(CH_2)_3\overset{+}{N}Me_3$		16.7	38.0		1.1×10^{-5} M
		±2.1			
		(4)	(1)		
β-pyridyl$CH_2CH_2\overset{+}{N}Me_3$		4.1	5.2	8.3	5×10^{-6}
		±0.3	±0.4	±0.3	
		(3)	(3)	(3)	

Mean values are shown ± s.e., with the number of results in parentheses. Values marked with an asterisk were obtained by Barlow & Franks (1971).

used on the rat diaphragm was about 5×10^{-5}M, compared with concentrations of 2 or 3×10^{-6}M used to stimulate the guinea-pig ileum (Table 3). The results suggest then that *m*-hydroxyphenylpropyltrimethylammonium and coryneine should have considerable specificity for ganglia unless there are large differences in species sensitivity (which is possible with desensitizing neuromuscular blocking agents). In concentrations up to 3×10^{-5}M, *m*-hydroxyphenylpropyltrimethyl was without effect on guinea-pig strip preparations, free from parasympathetic ganglia, nor did it affect the responses to carbachol (Fiona Roberts, personal communication).

Ganglion-blocking agents

Estimates of log affinity constant with *m*-hydroxyphenylpropyltrimethylammonium as agonist are shown in Table 6. With several of the compounds it was only possible to obtain dose-ratios less than 10; with higher concentrations, the antagonism was unsurmountable, presumably because the compounds blocked the postganglionic receptors. A few compounds also caused the development of spontaneous activity in the resting ileum. The results in Table 6 also include estimates of relative neuromuscular blocking activity on the rat diaphragm. These are only very approximate, because

Table 4 Pressor activity in pithed rats.

$R-\overset{+}{N}Me_3$	*log mean epmr*	
R=		
pm-$(HO)_2C_6H_3CH_2CH_2CH_2$- (coryneine)	$\bar{1}.8$	(5)
p-$H_2NC_6H_4CH_2CH_2$-	0 (standard)	
m-$HOC_6H_4CH_2CH_2$- (leptodactyline)	0.0	(6)
m-$HOC_6H_4CH_2CH_2CH_2$-	0.0	(11)
Suberylcholine	0.0	(5)
pm-$(HO)_2C_6H_3CH_2CH_2CH_2$-	0.1	(6)
β-pyridyl-CH_2-	0.1	(7)*
β-pyridyl-CH_2-CH_2-	0.15	(10)*
$C_6H_5CH{=}CH{-}CH_2$- (trans)	0.2	(4)
o-$H_2NC_6H_4CH_2CH_2$-	0.5	(9)
m-$HOC_6H_4CH_2$-	0.6	(9)
m-$H_2NC_6H_4CH_2CH_2$-	0.6	(10)
Dimethylphenylpiperazinium (DMPP)	0.7	(5)*
Nicotine	•	
Phenylcyclopropyl- (trans)	•	
m-$MeC_6H_4CH_2CH_2$	•	
m-$FC_6H_4CH_2CH_2$	•	
p-$H_2NC_6H_4CH_2CH_2CH_2$	•	
p-$H_2NC_6H_4CH{=}CHCH_2$ (trans)	•	
$C_6H_5C{\equiv}CCH_2$	•	

The standard stimulant was *p*-aminophenethyltrimethylammonium and the figures show the logarithm of the mean equipotent molar ratio (epmr) with the number of comparisons in parentheses. An asterisk indicates that stimulant activity was accompanied by desensitisation which in some instances made it impossible to obtain a quantitative result.

The following (trimethylammonium) compounds were blocking agents without themselves producing a rise in blood pressure:

p-$H_2NC_6H_5CH_2$-, *p*-$MeC_6H_4CH_2$-, *p*-$FC_6H_4CH_2$-, *o*-$MeC_6H_4CH_2CH_2$-, *p*-$MeOC_6H_4CH_2CH_2$-, *pm*-$(MeO)_2C_6H_3CH_2CH_2$-, *p*-$FC_6H_4CH_2CH_2$-, *p*-$MeC_6H_4CH_2CH_2CH_2$-, *p*-$MeOC_6H_4CH_2CH_2CH_2$-, *pm*-$(MeO)_2C_6H_3CH_2CH_2CH_2$-, β-pyridyl$CH_2CH_2CH_2$-, $C_6H_5CH{=}CHCH_2$- (*cis*).

the result is greatly affected by the order in which the compounds are given. They do indicate, however, that although high ganglion-blocking activity is often associated with high neuromuscular blocking activity, with many chloro compounds for instance, though there are others, such as *p*-bromobenzyldimethylamine, *o*-methoxyphenylpropyltriethylammonium, phenylpropyl- and phenylbutyldiethylamine, where there appears to be appreciable specificity for ganglia.

Affinity constants for postganglionic receptors are shown in Table 7.

Ionization

Although some of the compounds studied were tertiary bases, these are likely to be largely ionized at body pH. Barlow, *et al.* (1969) recorded a pK_a of 9.14 for phenethyldiethylamine in 40% v/v ethanol at 25°C and we have obtained a value of 9.3 for this compound in pure water at 25°C. The corresponding values for the benzyl, phenylpropyl and phenylbutyl diethylamines were 9.1, 9.6 and 9.8, respectively.

Discussion

The results indicate the potential value of *m*-hydroxyphenylpropyltrimethylammonium and 3,4-dihydroxyphenethyltrimethylammonium (coryneine) as ganglion stimulants in *in vitro* tests. Further work is needed to see how far their usefulness *in vivo* may be limited by neuromuscular blocking activity and it seems worth testing their tertiary dimethylamino analogues to see if these retain ganglion-stimulant activity but lack neuromuscular blocking properties. The change from triethylammonium to diethylamino effectively restricts blocking activity to ganglia (see below) so it is possible that the same may occur with stimulants.

Table 5 Neuromuscular blocking activity of ganglion-stimulants on the isolated phrenic-nerve-diaphragm preparation.

(+)-Tubocurarine chloride	300
p-$H_2NC_6H_4CH_2CH_2\overset{+}{N}Me_3$	300
pm-$(HO)_2C_6H_3CH_2CH_2\overset{+}{N}Me_3$ (coryneine)	250
m-$HOC_6H_4CH_2CH_2CH_2\overset{+}{N}Me_3$	240
m-$HOC_6H_4CH_2CH_2\overset{+}{N}Me_3$ (leptodactyline)	230
Decamethonium iodide	230
$C_6H_{11}CH_2CH_2CH_2\overset{+}{N}Me_3$	180

The maximum score in the test is 300, indicating complete block in 5 min at all three dose-levels (0.04 and 0.02 ml of 10^{-1}M and 0.1 ml of 10^{-2}M added to a 20 ml bath). Each compound was tested on two preparations. Even though the interval between doses was at least 15 min, the effects of a dose depend greatly on those of previous doses and the error in the score is likely to be at least ± 50.

Table 6 Ganglion-blocking activity on guinea-pig ileum and neuromuscular blocking activity.

	X-C_6H_4-$(CH_2)_n$-R			
n= 1				
R= $\overset{+}{N}Et_2H$	o	m	p	H
X = Cl	5.57 (8) *	5.50 (4)	4.69 (1) *	5.25 (6)
	20	*100*	*25*	*0*
Br		5.67 (5) *	6.42 (4)	
		0	*75*	
OH			4.96 (5)	
			0	
R= $\overset{+}{N}Et_3$				
X = Cl		5.35 (5)	5.53 (2)	5.82 (6)
		5	*80*	*10*
Br			5.86 (7)	
			135	

Table 6 *continued*

$X\text{-}C_6H_4\text{-}(CH_2)_n\text{-}R$

	o	m	p	H
n= 2				
R= $\overset{+}{N}Et_2H$				
X= Cl	5.7**			5.64 (6) *0*
Br			5.64 (4)	
R= $\overset{+}{N}Et_3$				
X= Cl		6.15 (4) *220*	6.26 (4) *200*	5.99 (4) *45*
NO_2		4.70 (4) *15*		
n= 3				
R= $\overset{+}{N}Et_2H$				
X= Cl	6.11 (6) *60*	6.04 (4)* *90*	5.4** *80*	6.06 (5) *0*
R= $\overset{+}{N}Et_3$				
X= Cl		6.64 (8) *210*	6.30 (3) *190*	6.19 (8)* *130*
OH		5* *85*	4.33 (3)* *50*	
OMe	6.28 (5) *55*	5.96 (4)		
n= 4				
R= $\overset{+}{N}Et_2H$				6.27 (5) *10*
$\overset{+}{N}Et_3$				6.86 (5)* *140*
n= 5				
R= $\overset{+}{N}Et_2H$				5.68 (4)* *25*
$\overset{+}{N}Et_3$				7.03 (5)* *100*
$PhCH{=}CHCH_2NMe_3$ (cis)		4.11 (3)*		
$p\text{-}BrC_6H_4CH{=}CHCH_2NEt_2$ (trans)		5.81 (6)*		
$C_5H_9(CH_2)_3NEt_2$		5.52 (6)		
$C_6H_{11}(CH_2)_3NEt_2$		6.39 (3)*		
Pempidine		6.09 (5) *0*		
(+)-Tubocurarine chloride		5.55 (4) *300*		
Decamethonium iodide		*230*		

Mean estimates of log affinity constant are shown with the number of estimates in parentheses. The standard error was usually less than 0.1 log units. Results for compounds with which it was only possible to obtain dose-ratios less than 10 are marked with an asterisk; the others are based on dose-ratios of up to 20. Some compounds produced a marked disturbance of the ileum which resembled spontaneous activity and this is indicated by a double asterisk. The number in italics is the average score in the tests on the rat diaphragm (maximum = 300).

Relationships between structure and activity appear to be complex but this is partly because the results in the experiments on the ileum are complicated by actions at postganglionic receptors. The optimum chain length, for instance, is apparently two methylene groups in the *p*-amino- and 3:4-dihydroxy compounds but three methylene groups in the *m*-hydroxy- compounds. It is also apparently two methylene groups in the unsubstituted compounds, but this is partly due to postganglionic stimulant activity in the phenethyl compound. The further possible complication of postganglionic blocking activity makes it impossible to say whether ganglion-stimulant acitivity increases or decreases with side-chains longer than three methylene groups.

Substituents other than hydroxyl or amino markedly reduce activity and there is no reason to link activity with the electronic effects of substituents. The aromatic nature of the ring may not, in fact, be essential because the cyclopentyl compound has some stimulant activity, though the results are complicated by its weak postganglionic stimulant activity (it appears to be a partial agonist at postganglionic receptors). It has been suggested that activity might be enhanced by the presence of rigid features in the side chain (Wong & Long, 1962; Kirkendol, Woodbury & Elko, 1972) but our results do not support this. *cis*-Phenylpropenyltrimethylammonium is a weak blocking agent and although the *trans* isomer, and *trans*phenylcyclopropyltrimethylammonium have some activity (Table 2), this is partly postganglionic. The present results do not, however, rule out the suggestion (Barlow, *et al.* 1969) that activity 'is associated with the presence of substituents which can interact with water molecules which may be involved in the action of the drug at the receptor'.

With the ganglion-blocking agents it is possible to observe the effects of substituents on affinity for ganglionic and postganglionic receptors (Table 8) and it is remarkable how often the introduction of a substituent reduces affinity and how seldom it enhances it to any great extent. It is also striking that the effects of substituents on affinity are qualitatively similar even though the compounds

Table 7 Affinity for postganglionic receptors.

			$Ph(CH_2)_nNEt_2$		
n=	1	2	3	4	5
	3.818 (3)	4.253 (4)	4.809 (4)	4.980 (6)	5.595 (6)
			$X\text{-}C_6H_4\text{-}(CH_2)_3R$		
X=Cl			o	m	p
$R=\overset{+}{N}Et_2H$			4.894 (3)	5.13* (2)	4.75* (1)
$R=\overset{+}{N}Et_3$			5.51* (3)	5.427 (5)	5.243 (4)
			$m\text{-}XC_6H_4(CH_2)_n\overset{+}{N}Et_3$		
n=1					
X=Br			5.190 (4)		
H_2N			4.365 (4)		
n = 2					
X=Cl			5.179 (5)		
O_2N			4.430 (4)		
HO			4.357 (4)		
n = 3					
X=HO			5.081 (4)		
MeO			4.789 (4)		
$C_5H_9(CH_2)_3NEt_2$			4.785 (5)		
$C_6H_{11}(CH_2)_3NEt_2$			5.333 (5)		
(+)-Tubocurarine chloride			about 5.3 (3)		

Values of log affinity constant are shown with the number of estimates in parentheses. The standard error of the estimates was usually about 0.04 log units and never exceeded 0.10 log units. Compounds which disturbed the resting intestine are indicated by an asterisk. The value for (+)-tubocurarine chloride is only approximate; the compound did not appear to act competitively.

Table 8 Effects of changes in structure on affinity for ganglionic and postganglionic receptors.

$X\text{-}C_6H_4\text{-}(CH_2)_n\dot{N}Et_3$

	logKg	*logKpg*	Δ *g*	Δ *pg*
n= 1				
X= H	5.25	4.83		
m− Br			+0.42	+0.36
m− H_2N				−0.47
n= 2				
X= H	5.99	4.95		
m− Cl			+0.16	+0.23
m−O_2N			−1.29	− 0.52
m−HO				−0.59
n= 3				
X= H	6.19	5.18		
m−HO			−1.2	−0.10
m−MeO			−0.23	−0.40

$X\text{-}(CH_2)_3R$

R = $\dot{N}Et_2H$				
X = H	6.06	4.81		
o-Cl			+0.05	+0.08
m-Cl			−0.02	+0.32
p-Cl			−0.66	−0.06
R = $\dot{N}Et_3$				
X = *H*	6.19	5.18		
o-Cl				+0.38
m-Cl			+0.45	+0.24
p-Cl			+0.11	+0.06

Values of log K are shown for the unsubstituted compounds and the columns marked Δ show the effect produced by the substituent. The subscripts g and pg refer to values for ganglionic and postganglionic receptors, respectively.

have usually about ten times the affinity for ganglionic receptors as they have for postganglionic ones (this can be seen from the distribution of + and − signs in Table 8). The affinity of the cyclopentyl and cyclohexyl compounds (Table 6 and 7) indicates that the aromatic ring is not essential for binding and there is no reason to suppose that the effects of a group on affinity are simply related to its effects on electron distribution. The effects of a substituent on affinity are different in the different series of compounds and the results support the view (Barlow, *et al.* 1969) that with these compounds affinity and substituent cannot be fitted into an equation such as those described by Hansch (Hansch, Maloney, Fujita & Muir, 1962; review by Tute, 1971).

The considerable specificity of the blocking agents for ganglionic receptors is illustrated for the unsubstituted compounds in Figure 1. Phenylbutyltriethylammonium has some neuromuscular blocking activity but this is much less in its analogous tertiary base, phenylbutyldiethylamine. This latter compound appears to be a ganglion-blocking agent with considerable activity and specificity, easy to make, and potentially capable of crossing membranes, such as those of the gastrointestinal tract or the 'blood-brain barrier'. Phenylpropyldiethylamine is a weaker ganglion-blocking agent that may be even more specific (Table 6). The properties of these compounds appear to be worth investigating in greater detail.

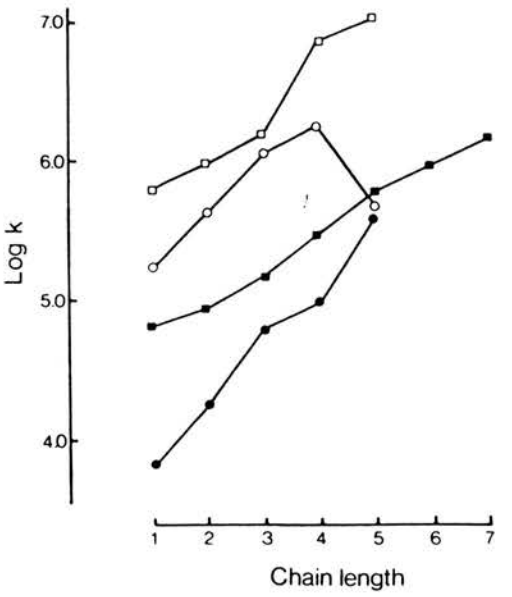

Fig. 1 Values of log K for postganglionic receptors (■,●) and for receptors in ganglia (□,○) are plotted against the number of methylene groups (chain length) for the compounds $Ph(CH_2)_n\overset{+}{N}Et_3$ (■ and □) and $Ph(CH_2)\overset{+}{N}Et_2H$ (● and ○). Note the greater affinity of both types of compound for ganglionic receptors and the considerable affinity of the tertiary bases, particularly of phenylbutyldiethylammonium (chain length = 4).

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THE EFFECTS OF PROSTAGLANDIN E_1 AND SODIUM MECLOFENAMATE ON BLOOD PRESSURE IN RENAL HYPERTENSIVE RATS

DANIEL MCQUEEN and KEITH BELL

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, Scotland

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Conflicting evidence exists regarding the ability of PGE_1 to normalize blood pressure in renal hypertensive rats. We performed experiments to determine the effect of PGE_1 (15 μg/kg i.p. daily for 3 weeks) in renal hypertensive Wistars and found no significant change in systolic pressure. A higher dose (150 μg/kg i.p.) lowered pressure after 14 days of treatment, but not back to control levels. Further investigations are required to establish the mechanism whereby PGE_1 evokes this fall.

To test the hypothesis that endogenous prostaglandins have a hypotensive function in renal hypertension, experiments were performed using sodium meclofenamate to inhibit prostaglandin biosynthesis. In chronic hypertensive rats the drug had no significant effect, while in the acute phase of renal hypertension there was a dose-dependent inhibition of the pressure rise. The possibility is suggested that prostaglandins may initiate or sustain the acute phase of renal hypertension in rats.

Sodium meclofenamate | Renal hypertension | Prostaglandins | Blood pressure | Rats | PGE_1

1. Introduction

It has been suggested that human essential hypertension as well as experimental renovascular and renoprival hypertension may be a result of an absolute or relative deficiency of renal prostaglandins (Lee et al., 1965; Lee, 1967). There is a lot of evidence in favour of this suggestion. Prostaglandins can lower blood pressure (Bergström, 1967), they occur in the kidney (Lee et al., 1966), and renal ischaemia provokes their release (McGiff et al., 1970). The renal prostaglandin content increases in experimental renal hypertension (Somova, 1971a) and there are reports that chronic administration of PGE to renal hypertensive animals lowers their blood pressure to normotensive levels (Muirhead et al., 1967, 1968; Somova and Dochev, 1970a; Somova, 1972). The latter evidence is, however, equivocal since Wendling and DuCharme (1974) found that PGE_1 did not decrease arterial blood pressure in renal hypertensive rats.

Somova (1972) and Wendling and DuCharme (1974) used slightly different experimental techniques in arriving at different conclusions regarding the ability of small doses of PGE_1 to normalize blood pressure in renal hypertensive rats. For example, the former used Wistar rats while the latter used Sprague—Dawleys. If was conceivable that the failure of Wendling and DuCharme to confirm Somova's findings stemmed from the slight procedural differences. We decided that further investigations on the effect of PGE_1 in rat renal hypertension were warranted, the object being to resolve the conflicting evidence regarding the abiability of PGE_1 to normalize blood pressure in renal hypertensive rats. Somova's techniques were followed as far as possible, the one major

difference being that we used male Wistars whereas Somova used an unspecified mixture of males and females.

We also tested the hypothesis that endogenous prostaglandins are involved as hypotensive agents in renal hypertension by using sodium meclofenamate to inhibit their biosynthesis (see Flower, 1974 for a review on prostaglandin synthesis inhibitors). If endogenous prostaglandins have important antihypertensive functions, inhibition of their synthesis should lead to a further rise of arterial blood pressure in established hypertension, or accelerate the rise in blood pressure during the acute phase of renal hypertension.

From our results we suggest that the possibility be further examined that increased prostaglandin levels, or an imbalance between the various prostaglandins, might directly or indirectly contribute to the acute phase of renal hypertension in the rat.

2. Materials and methods

2.1. Animals

Wistar rats (♂ and ♀) weighing 150—180 g (2 months old) were used in the experiments. They were individually coded and housed 5 to a cage in a room with controlled temperature (21°C), humidity (R.H. 50%) and lighting (12 h light (08.00—20.00) alternating with 12 h darkness). Water and Oxoid modified 41B diet were provided ad lib and the rats were weighed once a week.

2.2. Blood pressure measurement

Measurements were made in the same cage order and at the same time of day (09.15—14.00), generally twice weekly. Systolic blood pressure was measured indirectly by using the tail cuff method, pulsations of the tail artery in prewarmed rats being monitored by a photoelectric detector (Huntingdon Instruments Rat Blood Pressure Monitor) and recorded on a Devices M2 recorder together with cuff pressure.

The rats were prewarmed by placing them in a glass-doored incubator at 35°C for 20 min. The tail cuff was then applied while the rat was loosely restrained in a heated (37°C) holder and 5 consecutive readings of systolic pressure obtained. Pressure was determined by rapidly increasing pressure in the cuff until the tail pulse disappeared, then gradually releasing the pressure at a reproducible rate over 10—15 sec and noting the pressure at which the pulsations first reappeared. This was taken as the systolic blood pressure (see fig. 1) and the last 3 of the 5 measurements, if consistent, were averaged to provide the systolic reading. Occasionally a rat became agitated and it was not possible to obtain a reading on that particular day.

Consistent readings were obtained by following a fixed routine and ensuring that the same person performed the measurements at the same time on each occasion. It was important also to ensure that the tail cuff was consistently located on the same part of the tail and that extraneous noise was kept to a minimum. The rats were trained in the routine by making measurements for 3—4 weeks before

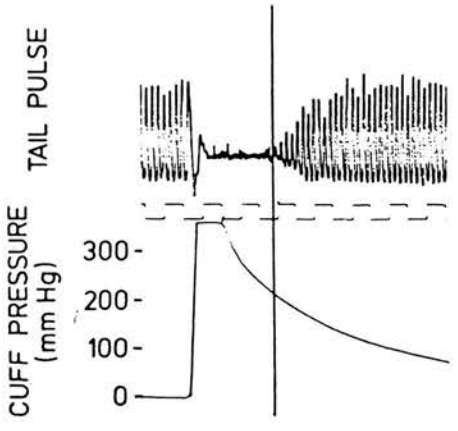

Fig. 1. Sample trace showing tail artery pulsations together with tail cuff pressure. The pressure corresponding with the reappearance of pulsations is marked and this is taken as systolic blood pressure. 1 sec time marker.

the first operation. We established that estimates of arterial blood pressure obtained from conscious rats by the tail cuff method were not significantly different from those obtained concomitantly via an indwelling carotid artery catheter, inserted under halothane anaesthesia 5 days prior to the measurements. It is necessary to use an indirect method of estimating blood pressure for long-term experiments because of the problem of keeping indwelling catheters patent over long periods. The preoperative systolic pressures obtained in the present experiments are similar to those previously reported for rats (Weeks and Jones, 1960; Finch, 1971; Wendling and DuCharme, 1974).

2.3. Operative procedure

Rats were anaesthetized with halothane and the left kidney exposed by a retroperitoneal incision. The renal artery and vein were separated and a sliver clip (6 mm × 2 mm × 0.25) was placed around the artery and squeezed until the renal artery was slightly constricted (mean internal diameter of clip 0.30 mm). The incision was sutured and the animal injected with benzathine penicillin (22.5 mg i.m.).

After an interval of 2 weeks the right renal artery was similarly clipped. Following recovery from the operations the blood pressure of most of the rats gradually increased. Hypertensive rats were arbitrarily defined as being those with a systolic blood pressure in excess of 150 mm Hg.

Sham operations involved procedures essentially similar to those described, excepting that the renal artery and vein were left untouched — the renal arteries were not clipped.

2.4. Drug administration

The rats were arranged in groups of 6—16 and drugs administered by daily i.p. injections, the injections being made at the same time each day (approx. 15.00 h).

Drug solutions were prepared and coded by a colleague such that we were unaware of which drug was being administered to a particular group until the experiment had been concluded. This was to minimise the influence of any operator bias.

2.5. Drugs used

PGE_1 and sodium meclofenamate were investigated. The former was injected at 2 dose levels, namely 15 μg/kg and 150 μg/kg. To assist solution, 0.25% ethanol was added to the weaker solution and 0.5% to the stronger. The same concentration of ethanol was present in the saline controls, saline (sterile 0.9% w/v aqueous sodium chloride) being the vehicle for the prostaglandin. Sodium meclofenamate (5 mg/ml) was also dissolved in saline.

All the drug solutions were made up to contain in 1 ml the dose to be injected per kilogram of body weight. Prior to each injection the rats were weighed and then injected with the appropriate amount of solution. When not in use the solutions were kept at −20°C, and biological activity of the PGE_1 was checked by assaying samples of the solutions about one week after the experiment had finished. All the solutions were of comparable potency when assayed against freshly prepared PGE_1, using the depressor effect on an anaesthetized rat's blood pressure as the test system, and U.V. analysis showed only traces of PGA_1 in the PGE_1 injection solutions.

2.6. Data analysis

Comparison of data from 'control' and 'treated' groups for any given measurement was made by *t*-test. The null hypothesis was rejected if $p < 0.05$ and the difference between groups described as statistically significant. In some experiments the same group provided its own control values by the comparison of pre-injection values with those obtained during the drug study.

3. Results

3.1. Controls

The first experiment undertaken involved 6 male Wistar rats, the object being to determine how blood pressure varied with changes in age, weight and tail diameter, and also to test the reliability of the blood pressure measuring technique.

Data obtained are given in table 1 from which it can be seen that quite consistent systolic blood pressure readings were obtained despite the physical changes in the rats. The experiment also established that neither the stress of the operation nor that of repeated blood pressure measurements had any noticeable effect on blood pressure.

3.2. PGE_1

2 groups of rats were made hypertensive and 3 months after the first operation daily i.p. injections were commenced. Blood pressure was measured twice weekly. For the first 5 days both groups received saline in order (a) to provide control values for subsequent statistical tests, (b) to show what influence the stress of the injection procedure had on blood pressure, and (c) to act as a control for subsequent injections since both groups were to be injected with solutions containing low concentrations of alcohol.

Following this period of saline injections, one group of rats received 15 μg/kg PGE_1 while the other group received the same volume of drug vehicle. The results obtained are shown in fig. 2. As there was no significant change in the blood pressure of either group after 20 days of treatment, the dose of PGE_1 was increased to 150 μg/kg and the injections continued for a further 21 days. A fall in blood pressure was observed in the PGE_1-treated group, this fall being statistically significant from the fourteenth day after increasing the dose of PGE_1. The mean blood pressure was, however, still significantly higher than the pre-operation control values and had not been normalized. 3 days after stopping the injections the pressure of the PGE_1-treated group was still significantly reduced, although by the eighth day it was not significantly different from pre-injection values. There was no significant change of mean blood pressure in the vehicle-treated group.

There is a range of systolic pressures in any group of hypertensive rats, and it is possible to subdivide the group into those with 'moderate' hypertension and those with 'severe' hypertension. The result of doing this for the PGE-treated group is shown in table 2 and the data suggest that the change in blood pressure is not related to the degree of hypertension since it is similar for both sub-divisions.

3.3. Sodium meclofenamate

Sodium meclofenamate was used to investigate the role of endogenous prostaglandins in renal hypertension (see the Introduction).

In the first series of experiments a control

TABLE 1

6 male Wistar rats — sham-operated. Mean systolic blood pressure and body weight before and after the operations.

	Pre-op.	Days after first sham operation				
		25	46	95	127	141
Systolic B.P. (mm Hg)	138	131	141	130	135	133
Body weight (kg)	0.23	0.32	0.35	0.43	0.44	0.45

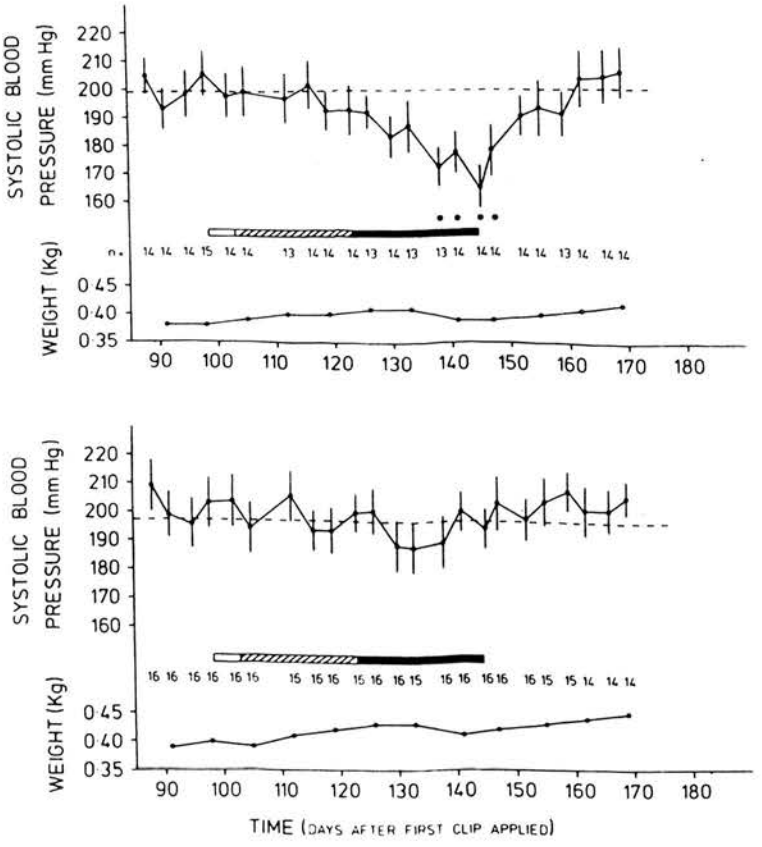

Fig. 2. Male Wistar renal hypertensive rats. Systolic blood pressure (mean ± S.E.M.) and mean weight plotted against time. The upper panel shows the values before, during and after daily i.p. injections, saline being administered during the period indicated by the open rectangle, PGE$_1$ 15 μg/kg during the hatched rectangle and PGE$_1$ 150 μg/kg during the black rectangle. There were initially 15 rats in the group with a mean pre-operative control pressure of 132 mm Hg; one rat died during the study. The number of rats measured on any given day is shown and the dotted line represents the mean of the 3 measurements prior to commencing the injections. The lower panel shows the values of a control group of hypertensive rats which were injected concurrently with the drug vehicle. There were initially 16 rats in this group with a mean pre-operative control pressure of 131 mm Hg; 1 rat died during the study. The symbol ⊗ in the upper panel indicates values which were significantly different ($p < 0.05$, t-test), in the case of the first three from the mean pressure on day 102 (saline injection), while in the case of the last three symbols the difference is that between the two groups, the vehicle-injected animals serving as a control group.

group of 15 unoperated male wistars was dosed daily with 5 mg/kg i.p. of sodium meclofenamate for 31 days. There was no statistically significant change in the group mean blood pressure during the period of treatment.

3.4. Sodium meclofenamate in established renal hypertension

We used male and female Wistar rats of this study. The females were much more difficult to

TABLE 2

Results from the group of hypertensive rats treated with PGE_1 (fig. 2) rearranged to show the data in terms of 'severe' hypertension (systolic pressure > 200 mm Hg) and 'moderate' hypertion (systolic pressure between 150—200 mm Hg). There were 7 rats in each group and the hypertensive control value is the average of the three readings prior to commencing treatment with PGE_1.

Days after first operation	'Moderate' hypertension		'Severe' hypertension	
	B.P. (mm Hg)	Δ B.P.	B.P. (mm Hg)	Δ B.P.
Pre-op control	133	—	131	—
Established hypertension control	175	—	220	—
102	174	−1	222	+2
119	171	−4	213	−7
130	166	−9	200	−20
145	144	−31	186	−34

handle than were the males, but by being patient consistent readings were obtained from them. The experiment consisted of administering sodium meclofenamate (5 mg/kg i.p. daily for 23 days) to rats 3 months after the first operation and recording blood pressure. The results obtained are shown in fig. 3 from which it can be seen that there was a tendency for blood pressure to rise during the treatment period, although no statistically significant difference was observed. When the results were analyzed according to the sex of the rats, it was found that initially the pressure of the male rats fell while that of the females rose slightly. This difference was also not statistically significant.

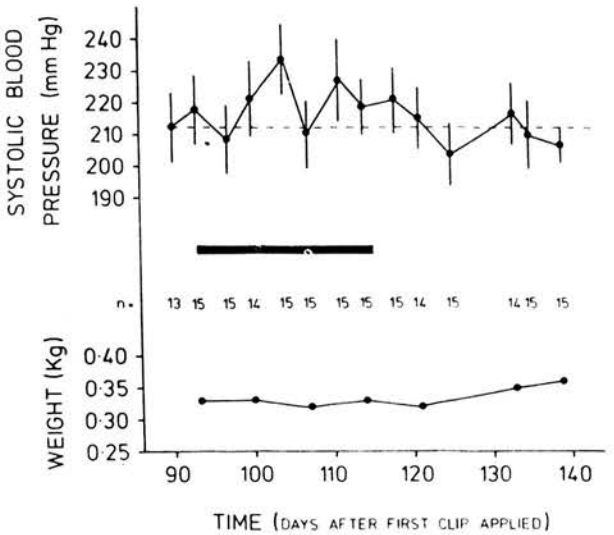

Fig. 3. Wistar renal hypertensive rats (8 females, 7 males) dosed during the period indicated by the black bar with sodium meclofenamate, 5 mg/kg i.p. daily. The systolic pressure (mean ± S.E.M.) is plotted against time as is the mean weight. The number of rats measured on any given day is shown and the dotted line represents the mean of the 3 measurements prior to commencing the injections.

However, in view of the possibility that different results might have been obtained with an all-male group, an additional experiment was performed in which a group of male renal hypertensive rats was similarly treated with sodium meclofenamate (5 mg/kg i.p. daily). The group used was composed of rats previously treated with PGE_1 (see fig. 2), it having been established by the earlier experiment that changes in prostaglandin levels, caused by dosing with PGE_1, resulted in blood pressure changes. The results obtained are shown in fig. 4 and it can be seen that no significant alteration in systolic blood pressure occurred during treatment with sodium meclofenamate. The tendency was for the pressure to fall, but this fall was not statistically significant. The rats' weight was slightly reduced by the end of the injection period, but this reduction was also not statistically significant. The all-male group thus responded similarly to the mixed group.

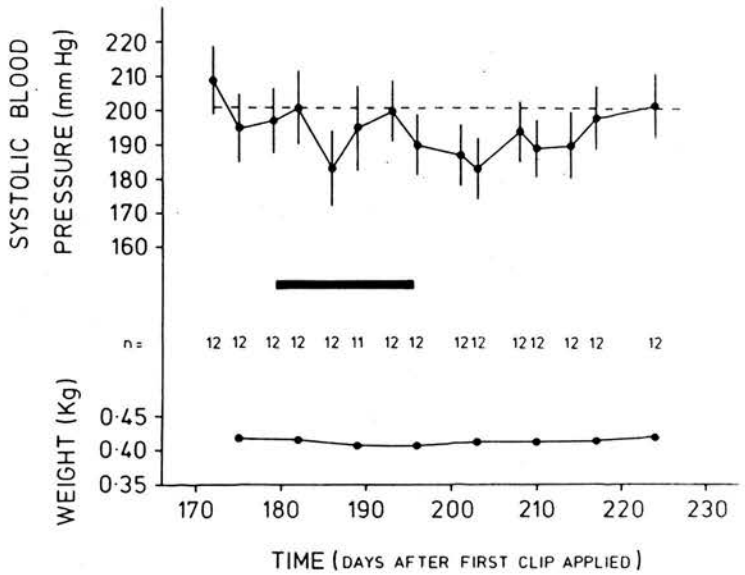

Fig. 4. Wistar renal hypertensive rats (12 males) dosed during the period indicated by the black bar with sodium meclofenamate, 5 mg/kg i.p. daily. Other details as for fig. 3.

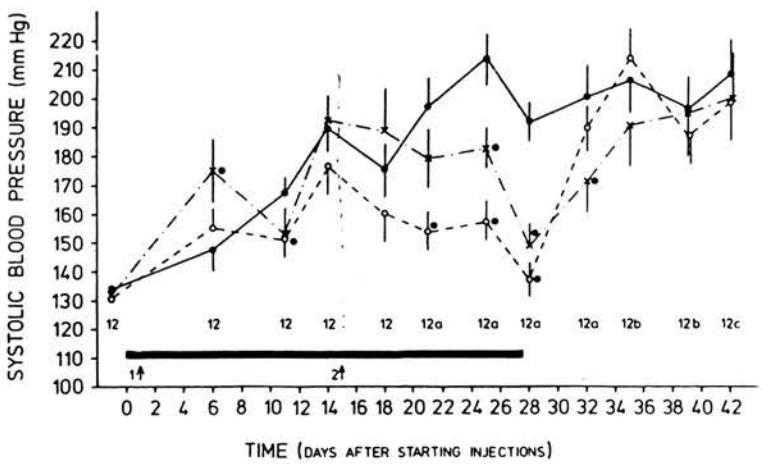

Fig. 5. The influence of sodium meclofenamate in the acute phase of renal hypertension in male Wistar rats. Daily i.p. injections during the period represented by the black rectangle of (×- - -×) sodium meclofenamate 0.5 mg/kg; (○- - - - -○) sodium meclofenamate 5 mg/kg; and (●———●) saline. The blood pressure (mean ± S.E.M.) is plotted against time and the number of rats measured in each group is shown (12), (a) meaning that 1 rat in the low dose meclofenate group had died, there subsequently being only 11 measured in that group, (b) that a rat in the high dose meclofenamate group had died and (c) that another died in this group, meaning there were only 10 survivors. The arrows indicate the days on which the first (1) and second (2) operations were performed, and control pressures (day 0) are the mean of the three measurements preceding the first operation. ⊗ indicates a significant difference ($p < 0.05$, t-test) between the mean pressure of the drug-treated group and that of the saline group on the same day.

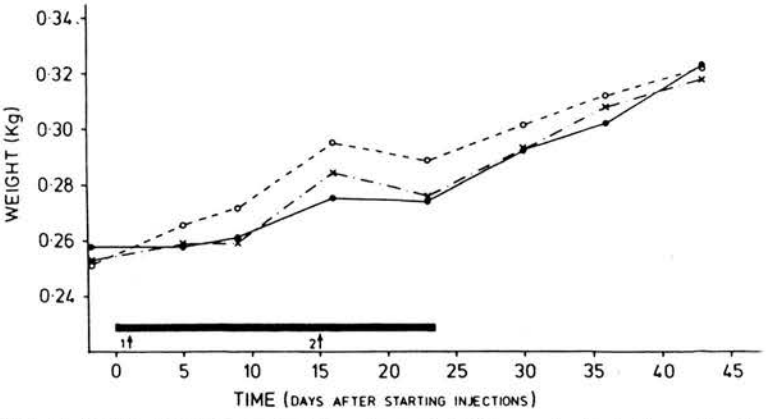

Fig. 6. Acute renal hypertension in male Wistar rats. Details as for fig. 5, this graph showing mean of the group weights against time.

3.5. Sodium meclofenamate in acute renal hypertension

A further experiment was performed in order to investigate the influence of sodium meclofenamate on blood pressure during the acute phase of renal hypertension. The blood pressure of 3 groups of young rats was measured in order to establish control levels, and daily i.p. injections were commenced the day before clipping the first renal artery and continued for 27 days, at which time they were stopped as the rats were losing weight. Blood pressure measurements were continued throughout the treatment period and for 2 weeks thereafter.

As in the previous experiments, the injections were made 'blind'. One group received saline, one sodium meclofenamate at 0.5 mg/kg, while the third group received sodium meclofenamate at a dose of 5.0 mg/kg. The results obtained are shown in fig. 5 from which it can be seen that during the period of treatment statistically significant differences were observed between group systolic pressures. The reduction in blood pressure, or the prevention in pressure rise in the meclofenamate-treated rats was dose-dependent and, in the case of the higher dose level, returned blood pressure to a level which did not differ significantly from the pretreatment controls. It should be noted that a significantly higher pressure was observed in the low dose group, as compared with the saline group, 5 days after the first operation.

When the injections were stopped the pressures of the drug-treated groups rapidly rose to reach the same level as the saline group within 7 days. The rise in pressure of the high dose group was very rapid and was associated with the death of two of the rats at this time.

Fig. 6 shows the mean weights of the groups. There was a slight fall in weight of the meclofenamate-treated rats after the second operation, but this was not statistically significant and the saline-treated group also stopped gaining weight as this time.

4. Discussion

We were unable to demonstrate any hypotensive effect of low doses of PGE_1 in chronic renal hypertensive rats, and to that extent our findings agree with those of Wendling and DuCharme (1974). These authors reported that PGE_1 at a dose of 150 μg/kg i.p. daily for 4 days in rats with established renal hypertension failed to cause any significant change in blood pressure. In contrast, Somova (1972) found that PGE_1, at the lower dose of 20 μg/kg i.p.

daily, normalized blood pressure within 5 days. Our results show no significant blood pressure change after administering PGE_1 at a dose of 150 μg/kg i.p. daily for 10 days. However, after 14 days of treatment with this higher dose, blood pressure fell significantly, although at no time did it return to control levels. Blood pressure returned to pre-injection hypertensive levels within 7 days of stopping the injections (see fig. 2).

It could be argued that in the present investigation the degree of hypertension was greater than that obtained by previous workers and that therefore the results are not comparable. The rise in pressure also occurred more rapidly, being evident within 10 days of clipping the first renal artery, whereas Somova and Dochev (1970a) reported that their rats were still normotensive 21 days after clipping the first renal artery. By grouping our rats according to their degree of hypertension, we established that those with 'moderate' hypertension (pressure levels similar to previous reports) and those with 'severe' hypertension responded to PGE_1 with similar decremental changes in systolic blood pressure (table 2). Since the response eventually elicited by PGE_1 appears to be unrelated to the degree of hypertension, comparison of our findings with those from experiments involving less severe hypertension, as judged from systolic blood pressure, appears valid.

It is also unlikely that the fall in blood pressure of the PGE_1-treated group is directly caused by the slight, but not statistically significant, weight loss. The vehicle-treated group also lost weight, and yet their blood pressure did not fall. What causes the slight weight loss observed in the present investigation requires further study, but it may have been a reaction to the prolonged injection period of 46 days or the presence of 0.5% ethanol in the injection solution.

One might anticipate that low doses of PGE_1 administered i.p. would be slowly absorbed, probably into the hepatic portal system (Lukas et al., 1971), and therefore rapidly cleared from the blood by liver and lung metabolism (Dawson et al., 1968; Ferreira and Vane, 1967). The implication would be that only minimal quantities of PGE_1 reach the systemic circulation. Perhaps these minimal amounts, or metabolites of PGE_1, are responsible for the chronic fall in blood pressure? Experiments in conscious normotensive rats (Somova, 1971b) and in pentobarbitone-anaesthetized rats (McCaig and McQueen, unpublished observations) demonstrated that PGE_1 at a dose of 15 μg/kg i.p. causes a transient lowering of arterial blood pressure, the effect lasting 20—40 min. Assuming that this effect arises from actions on the systemic vasculature, it is evident that either PGE_1 on i.p. administration can reach the systemic circulation (perhaps by overloading the prostaglandin inactivating mechanism, which might not be very efficient in the rat according to Papanicolaou and Meyer, 1972) or that a metabolite is active in lowering blood pressure. A cumulative effect of the acute blood pressure changes is not a likely explanation of the chronic blood pressure reduction which eventually develops since doses of 100 μg/kg PGE_1 i.p. daily for 9 months do not affect blood pressure in normal rats (Somova et al., 1973), and nor does PGE_1 have significant hypotensive effects in rats with sodium chloride hypertension (Somova and Dochev, 1971).

The mechanism(s) responsible for the latent blood pressure lowering effect of exogenous PGE_1 (also described by Muirhead et al. (1968) using much higher doses (0.75—3.3 mg/kg s.c.) of PGE_2) and its physiological significance could not be deduced from our experiments and further work is needed to clarify the matter.

Since our results with low doses of PGE_1 were negative in that we failed to detect any hypotensive effect, we decided to employ another approach to investigating the question of whether or not prostaglandins play a role in renal hypertension, and this involved the use of sodium meclofenamate to inhibit prostaglandin biosynthesis. If endogenous renal prostaglandins have a hypotensive function, as has been proposed by Lee et al. (1965) and Muirhead et al. (1972), then inhibition of their

synthesis, there being little stored in the kidney (McGiff et al., 1974), should raise blood pressure to higher levels. However, whereas Davis and Horton (1972) reported a pressure rise in normotensive anaesthetized rabbits treated with indomethacin, our results showed very little change in the blood pressure of normotensive rats or of rats with established hypertension when they were treated with sodium meclofenamate (figs. 3 and 4). It has been reported (Somova et al., 1974) that treatment with aspirin or indomethacin (1 mg/kg/day orally for 6 days) lowers the elevated prostaglandin levels in the kidneys of renal hypertensive rats, but does not affect arterial blood pressure.

Prostaglandin release from the kidney following renal ischaemia (McGiff et al., 1970) might only be involved in lowering blood pressure during the acute phase of renal hypertension or, alternatively, the two-clip renal hypertensive rat with established hypertension may not be able to show blood pressure changes in response to alterations in prostaglandin levels because of damage to the vascular system as a result of the sustained high pressure or because of sodium volume dependency (Gavras et al., 1975). Either of these possibilities would explain why inhibiting synthetase activity in established renal hypertension has no significant effect on blood pressure. We decided therefore to examine the effect of sodium meclofenamate during the acute phase of renal hypertension. If the hypothesis that prostaglandins have a hypotensive role is correct, then inhibition of their biosynthesis during the acute phase of hypertension should cause the blood pressure to rise more rapidly.

The results we obtained (see figs. 5 and 6) showed a dose-dependent reduction of the pressure rise, this being particularly obvious following clipping of the second renal artery. There was a transient increase in pressure (above the control group value) in the rats treated with the low dose of meclofenamate 5 days after clipping the first renal artery. After the injections were stopped blood pressure in both drug-treated groups rapidly rose to a level comparable with that of the control group, thereby suggesting that sodium meclofenamate was acting by causing a dose-dependent inhibition of a system involved in raising blood pressure in renal ischaemia, or perhaps in sustaining the initial rise.

Interpretation of our findings depends upon the specificity of sodium meclofenamate as an inhibitor of prostaglandin biosynthesis. Meclofenamate was used in preference to indomethacin because it is readily soluble in water, is as potent when administered systematically as when given orally, and as it has a long plasma half-life it need only be administered once a day (Glazko, 1972; Kurtz and Fitzgerald, 1972). Following meclofenamate dosage in rats, drug levels are highest in the liver, kidney and plasma (Glazko, 1972). Since we were unable to find any data relating to the degree of inhibition of prostaglandin synthesis which would result from chronic treatment with sodium meclofenamate in rats, we resorted to selecting a dose based on the fact that meclofenamate is as potent or more potent than indomethacin on most synthetase preparations (Flower, 1974). Accordingly we used a dose of 5 mg/kg i.p. which, if equivalent to the same dose of indomethacin, should produce substantial inhibition of prostaglandin synthesis (Flower, 1974) without causing toxic effects (Kurtz and Fitzgerald, 1972). We did not in fact detect any toxic effects other than slight weight loss following prolonged treatment, and this was not statistically significant. In the acute hypertension experiment a dose of 0.5 mg/kg had effects which took longer to develop but which were qualitatively similar to those elicited by ten times the dose of meclofenamate. Recently Pugsley et al. (1975) reported that PGE_1 synthesis by rat renal medullary slices incubated with plasma from rats treated with indomethacin (5 mg/kg s.c. daily for 7 days) was reduced by 65%. However, without biochemical data we cannot be sure to what extent sodium meclofenamate was inhibiting prostaglandin biosynthesis and nor can we preclude the possibility that the dose used was affecting other enzyme systems (e.g. renin—

angiotensin). The same would still have been the case had we used indomethacin or aspirin.

If sodium meclofenamate was inhibiting prostaglandin synthesis specifically, our results could be interpreted as meaning either that prostaglandins directly or indirectly provoke the acute hypertension but have little influence on established hypertension, or that an imbalance of prostaglandins A, E and F arises such that blood pressure becomes elevated, possibly as a consequence of altered renal haemodynamics (McGiff et al., 1974). Renal prostaglandin synthetase activity in renal hypertensive rats initially falls and then rises (Grodzinska et al., 1974) and it is tempting to interpret our findings in terms of actions of meclofenamate on the production of renal prostaglandins. Malik and McGiff (1975) have recently provided evidence that prostaglandins of the E series modulate adrenergic transmission in the rat kidney and this results in an enhanced vasoconstrictor response to sympathetic nerve stimulation, the effect also being augmented by the prostaglandin precursor arachidonic acid and reduced by indomethacin. Laborit and Valette (1975) also found that arachidonic acid worsened hypertension in unilaterally nephrectomised rats treated with DOCA/NaCl, although they attributed this to central actions of PGE_2 formed from the arachidonic acid. There is thus other evidence to support the suggestion that prostaglandins may initiate or sustain the acute phase of hypertension in renal hypertensive rats.

Pugsley et al. (1975) recently reported that indomethacin causes a further rise in blood pressure of rats with only one renal artery clipped, the contralateral kidney being left untouched. This is contrary to our finding, but there is evidence that unilateral clip renal hypertension differs from that obtained with bilateral renal artery occlusion, or unilateral occlusion with contralateral nephrectomy (Gavras et al., 1975). Our results are not, therefore, directly comparable. It may be that the unilateral clip renal hypertensive rat will prove to be a more appropriate model in which to study the effects of altering prostaglandin levels on the course of renal hypertension, but the present investigation was concerned only with the two clip model because it was from this type of hypertensive rat that conflicting evidence had been obtained concerning the ability of prostaglandins to normalise blood pressure in established hypertension.

Interpretation of data from either of the types of hypertensive rat may well depend upon dissociating the acute from the chronic effects of altering prostaglandin levels and also in determining the specificity of drugs used to inhibit prostaglandin biosynthesis. With regard to the latter point, it is interesting to note than in rabbits arachidonic acid increases and indomethacin decreases plasma renin activity (Larsson et al., 1974). However, since it has been shown that the rat differs from the rabbit as far as the effects of indomethacin on responses elicited by sympathetic nerve stimulation are concerned (Malik and McGiff, 1975), the observations relating to renin levels in rabbits may have no relevance to the situation in the rat. Indeed Somova et al. (1974) were unable to detect any change in renin activity following treatment of hypertensive and normotensive rats with indomethacin, and Vander (1968) reported that prostaglandins have no effect on renin release. Despite this evidence to the contrary, it would seem worth investigating further the possibility that prostaglandin synthetase inhibitors affect, directly or indirectly via changes in prostaglandin levels, the renin—angiotensin system.

Further investigations into the nature and extent of prostaglandin involvement in the aetiology of bilateral clip renal hypertension in the rat seem warranted.

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Research Reports

NOXIOUS AND TACTILE INPUT TO MEDIAL STRUCTURES OF MIDBRAIN AND PONS IN THE RAT *

R. EICKHOFF, H.O. HANDWERKER, D.S. McQUEEN ** and E. SCHICK

II. Physiologisches Institut, Universität Heidelberg, 6900 Heidelberg (G.F.R.)

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SUMMARY

Response patterns of single units in the medial parts of the pons and midbrain were studied extracellularly in chloralose-anaesthetized rats, paralyzed with pancuronium.

The units were tested with natural skin stimuli including slight touch and hair-brushing in order to provide input from sensitive mechanoreceptors supplied by myelinated afferent fibres. Noxious pinch and controlled noxious radiant heat were also used to provide nociceptive input. The influence of these stimuli on unit activity was compared with that evoked by electrical stimulation of the coccygeal nerve, which excited either the A-fibres only or, with stronger stimuli A- and C-fibres. It has been shown that about half the units responding to electrical stimulation of the A-fibres exhibited a second peak of excitation with a latency of 100—300 msec when the stimulus strength surpassed C-fibre threshold. A high and statistically significant correlation has been found to exist between increased excitability induced by noxious heating and/or squeezing the skin on the one hand and the occurrence of a C-fibre response on the other hand.

From this evidence it is concluded that a large proportion of the units in the medial pons and midbrain receive nociceptive input from the skin and that this input is mediated predominantly by C-fibres.

When applying controlled radiant heat it has been found that the discharge frequencies of units responding to noxious input do not code the stimulus strength exactly, rather the units usually switch from a lower to a higher discharge frequency when noxious levels of stimulation are reached. The functional implications of this are discussed.

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INTRODUCTION

Several structures in the medial part of the midbrain might be involved in the processing of nociceptive signals. During the last few years it has been shown that electrical stimulation of structures in the periaquaeductal region of the midbrain can suppress nocifensive responses [22,24]. Furthermore, the same brain region has emerged as a structure of importance for the analgesic action of morphine [11,15,23,34,35]. From these findings the hypothesis has arisen that this region, particularly the central grey and the midbrain raphe nuclei, constitutes a "pain suppression centre" [23]. On the other hand, the central grey [27] and adjacent fields of the reticular formation [6, 27] might be involved in an ascending nociceptive system.

Anatomical [6,26,30] and evoked potential [20] studies provide evidence for somatosensory input to midbrain reticular and central grey neurones, but give little information on the type of the skin receptors involved. In the cat several single unit studies have been performed to investigate the proportion of units responsive to innocuous stimulation compared with those which respond predominantly or exclusively to noxious stimuli [2,4,9,10,12,31, 36]. Little is known, however, about these proportions in the rat, the species mainly employed in behavioural and pharmacological studies on the central nociceptive system. Even the results obtained in the cat are controversial, reports of the number of units responding to exclusively nociceptive input ranging from none [31] to almost 100% [36].

The aim of the present work was to study the characteristics of cutaneous input to midbrain central grey, raphe and reticular neurones in the rat with special reference to input from the tail, since the "tail-flick" is the most frequently studied nocifensive behaviour in this species. In subsequent studies we intend to study midbrain neurones in awake animals while testing the "tail-flick" reaction.

METHODS

Results were obtained from experiments on 21 male albino rats (Sprague–Dawley and Wistar strains) weighing between 250 and 350 g. The animals were initially anaesthetized with α-chloralose (70 mg/kg i.p.) with supplementary doses of pentobarbital (2 mg at intervals of approximately 3 h) being given i.v. A tracheotomy was performed and the right jugular vein was cannulated for drug administration. At the end of surgery the animals were paralyzed with pancuronium (s.d. 0.5 mg) and artificially ventilated.

The ECG was monitored throughout the experiment and no further recordings were attempted when signs of a cardiac conduction block appeared. In the case of a complete AV block the experiment was terminated. The body temperature was kept near 37°C by infrared radiation.

For controlled electrical stimulation the left main trunk of the coccygeal nerve was exposed at the tail base, cleared from the surrounding tissue for a length of about 15 mm, and slotted into a polyethylene tube containing

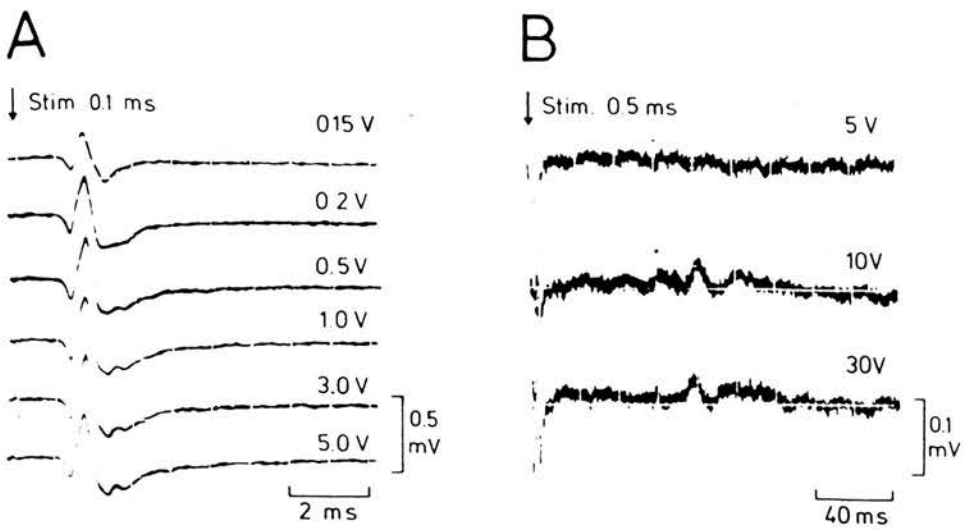

Fig. 1. Nerve volleys recorded with bipolar hook electrodes (platinum wires) from the coccygeal nerve on electrical stimulation of the nerve by the device described in Methods. A: stimulation above A-fibre threshold. Each trace 3—5 superpositions. Stimuli applied at a rate of 1/3 sec. B: stimulation below and above C-fibre threshold. Single shock C-fibre stimuli have been applied at a rate of 1/10 sec.

two platinum wires (0.1 mm in diameter, distance 8—10 mm) for stimulation. By this technique continuity in the nerve was maintained. The skin was sutured over the stimulating electrode in order to prevent its dislocation and to avoid drying of the nerve. In pilot experiments on 4 rats (not included in the experimental sample) we tested the stimulation arrangement by monitoring the nerve volley from the exposed nerve at a point 70—100 mm distal to the stimulating electrodes. Fig. 1 shows typical results.

A nerve volley appeared when 100—200 mV, 0.1 msec square pulse stimuli were delivered from an isolation unit (Digitimer). This volley represented action potentials travelling at about 35 m/sec (skin temperature 30°C). A stimulus strength of 3 V, 0.1 msec was always supramaximal for this A fibre compound action potential. It was not usually possible to separate clearly an $A\alpha,\beta$ from an $A\delta$ volley. (But see the small $A\delta$ component in Fig.1.) A C-fibre volley with conduction velocity between 0.8—2.0 m/sec appeared at a stimulus strength of about 8 V when 0.5 msec square pulses were used. It was saturated in all cases at 30 V, 0.5 msec stimulus strength.

In those experiments in which the aim was to record from midbrain units, we eliminated the second nerve preparation, necessary for recording the nerve volley, since it would have interfered with the natural skin stimulation. Instead we tried to monitor the focal potentials in the midbrain, which always had a threshold below a stimulus strength of 500 mV, 0.1 msec.

Natural stimulation of the skin receptors was performed by stroking the skin with a brush, by slight tapping and by strongly squeezing the skin with

a serrated forceps. In order to apply a more precise noxious stimulus, controlled radiant heat as used in previous studies on the cat [3,13,14] was employed. Briefly, a thermocouple was placed in good contact with the skin at a point on which the radiation of a halogen bulb was focused. The focal spot had a diameter of approximately 7 mm. The output voltage of the thermocouple was fed back to control the heating current of the bulb. A more detailed description of the method is given elsewhere [3].

For recording activity of mesencephalic and pontine units, the rat was placed in a stereotactic holder in the position specified by Koenig and Klippel [19]. Recordings were performed with glass-coated tungsten microelectrodes, having an exposed tip of 1—5 μm, which were inserted through a small hole drilled in the skull.

At the end of each track an electrolytic lesion was made. Usually only one and never more than two tracks were performed in a rat, this being in order to simplify histological reconstruction of the recording sites. Spike recordings were amplified with a conventional AC coupled preamplifier. All units which are documented in this study had primarily negative going peaks and a stable form and size throughout the testing. A window discriminator was used to distinguish unitary spikes from noise. Scatter displays of activity evoked by electrical nerve stimulation were constructed on line using the Z-input of a storage oscilloscope. Recordings were also stored in addition on magnetic tape. Responses to natural stimulation of cutaneous receptors were analyzed off-line constructing spike occurrence density histograms with a PDP-12 computer.

At the end of an experiment the animal was killed by an overdose of pentobarbital and was perfused through the abdominal aorta with 7% formaldehyde in saline. Frozen sections of the brain were cut at 30 μm thickness, and stained by the Nissl method in order to facilitate reconstruction of the electrode tracks.

RESULTS

(1) Sample of units tested

A total of 146 units have been studied between the stereotactic boundaries +700 μm and —1700 μm in the frontal plane, and between +/— 1000 μm lateral to the midline (stereotaxy according to the Koenig and Klippel atlas supplemented for the most caudal sections by the figures of Palkovits and Jacobowitz [19,32]).

Of these units 98 could be driven by electrical stimulation of the coccygeal nerve. Thus 65% of units received input from the tail. It is uncertain, however, if this proportion is representative of the neuronal population in this region, since we used a search stimulus (1.0—3.0 V, 0.1 msec) delivered to the coccygeal nerve when hunting for units. Non-responsive units with very low spontaneous activity might have escaped our notice. Of the 98 units driven by electrical stimulation 92 were also tested for excitation by "natural" skin stimulation. Seventeen of them (18%) could not be excited

by application of any kind of natural stimulus.

The spontaneous activity of the units in our sample was rather variable, ranging from 0.1 to more than 100 spikes/sec. Interval histograms (IHs) have been constructed of the spontaneous activity of some of the units having a level of spontaneous activity exceeding 20/sec. These IHs approximately resembled Poisson distributions in each case. Some units under study switched once or twice during the testing period from higher to lower activity or vice versa in the absence of experimentally induced stimulation. To avoid incorrect classifications we assessed only those units which were shown to be responsive to some type of natural stimulation and which responded consistently to at least two repetitions of natural stimulation. In no instance did we encounter discharges that were overtly correlated with respiratory rate or with heart rate.

(2) Responses to electrical stimulation of the nerve

All units described as "responsive to electrical stimulation of the coccygeal nerve" in this study had a determinable latency which could be demonstrated as a peak in a poststimulus time histogram constructed with a bin width of 10 or 100 msec from 16 repetitive stimuli (cf., Methods for repetition rate). All units which were found to fit the above criterion of responsiveness to electrical nerve stimulation responded to weak stimuli which activated A-fibres only. This response showed a latency of between 10 and 20 msec. The short latency found in the present study is in agreement with an evoked potential study in the rat midbrain [20] and indicates an oligosynaptic input from the tail. As can be seen from an example shown in Fig. 2 there is some recruitment between stimuli which just surpass the threshold for the largest A-fibres (Fig. 2A) and stronger stimuli (Fig. 2B), indicating an additional input from smaller myelinated fibres, perhaps in the Aδ range. In 5 out of 98 units the response to A-fibre stimulation consisted of an inhibition of spontaneous activity instead of an early excitation. In most of the other units the initial excitation was followed by an inhibition (cf., Fig. 2C) which in some units lasted more than 1 sec.

When the strength of stimulation was increased above the threshold for C-fibres, in about half of the units (43 of 83 units tested with C-fibre stimuli) a second peak of excitation could be seen (marked by an arrow in Fig. 2D). Only once did we record a second peak at a stimulus strength of 3.0 V, 0.1 msec, which might have been a rebound excitation due to input from A-fibres. In all other units the second peak required a stimulus intensity above 5.0 V, 0.5 msec. Since these high voltage and long duration stimuli only recruit very slow δ-fibres (cf., below 10 m/sec) and C-fibres beyond the fibre spectrum excited by weaker stimuli, the second excitation is presumably due to input from these fibre classes. A comparison of two examples (cf., Figs. 2 and 3) from two different units (recorded in different rats) illustrates the range of latencies of this second excitation.

In the case of Fig. 3, the central latency was only about 20 msec, if the C-volley in the peripheral nerve travelled at 1 m/sec. In Fig. 2D, however,

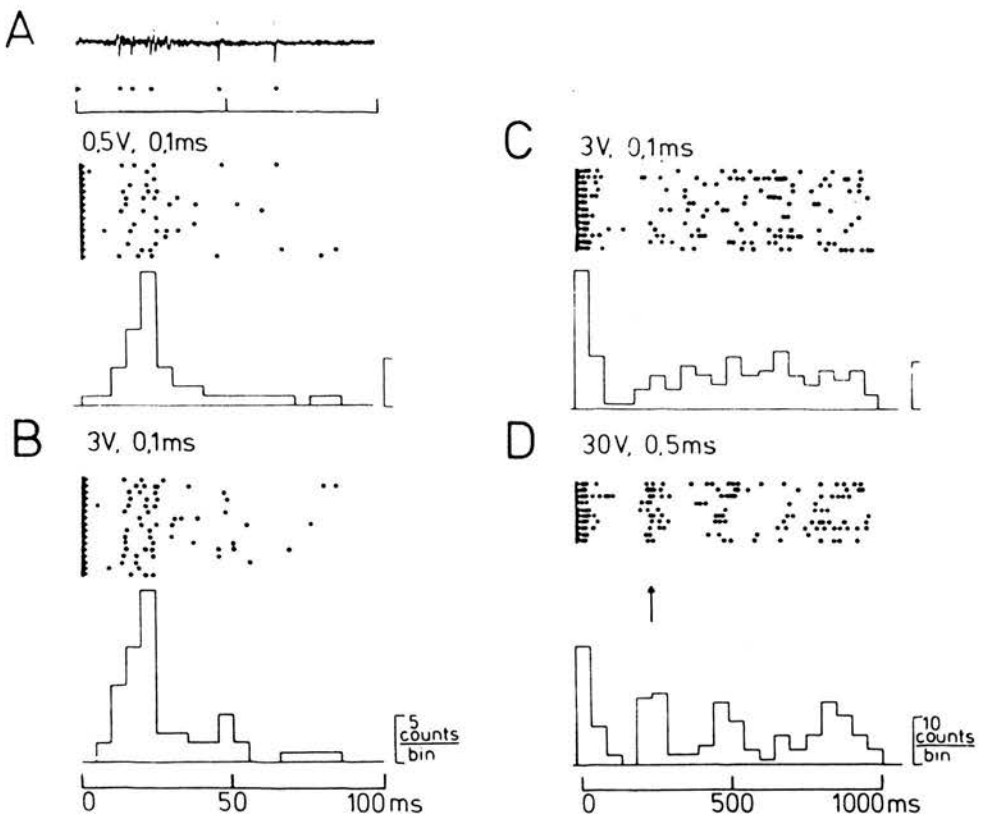

Fig. 2. Response patterns of a midbrain unit to electrical stimulation of different intensity. At the top: sample record together with a corresponding raster display. Below: raster displays and corresponding peristimulus time histograms of the discharges of the same unit to electrical stimuli of different strength. Calibrations in (A) as in (B), and in (D) as in (C), respectively.

conduction through the ascending system of the spinal cord seems to have been much slower. It is possible, however, that in this specimen a first part of an excitation by C input is masked by the preceding inhibition due to the A-fibre input.

When stimuli above threshold for C-fibres were delivered, about half of the units which responded to C-fibre stimulation exhibited an oscillation of excitation and inhibition lasting up to several seconds (cf., Fig. 2D). Those oscillations with 1—4 firing maxima/sec have also been observed during and after noxious heat stimulation and might be characteristic of responses to nociceptive input. Other units (cf., Fig. 3B), however, lacked those oscillations. We found no systematic difference of the distribution of the units with and without oscillations in different midbrain structures.

When stimulated above C-fibre threshold, 4 of the units which had been judged to be non-responsive on the basis of the above criterion nevertheless

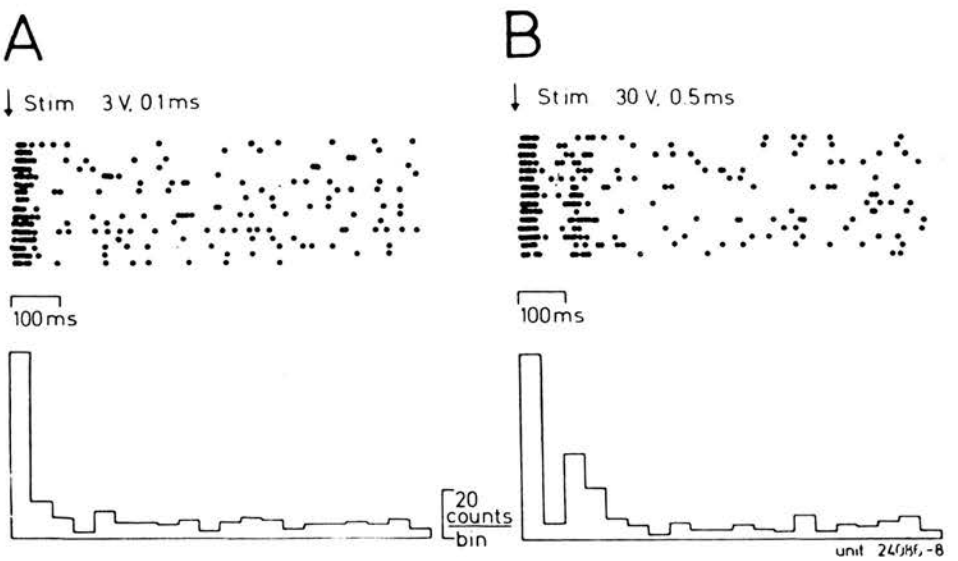

Fig. 3. Response pattern of a midbrain unit to electrical stimulation below (A) and above (B) the threshold for C-fibres. Dot displays as in Fig. 2. Each response sequence is shown as a horizontal line of dots. First stimulus at top. Peristimulus time histograms derived from the same data as dot displays. Stimulation frequencies: 1/3 sec in (A) and 1/10 sec in (B).

showed a more diffuse increase in activity lasting several seconds. An example of such a change in activity is shown in Fig. 4. These 4 units perhaps received input from C-fibres, but not from fast conducting A-fibres. No unit of this small sample, however, changed its firing pattern significantly during natural noxious stimulation.

When higher stimulus frequencies have been used, response patterns as shown in Fig. 4 became similar to the "wind up" phenomenon which can be

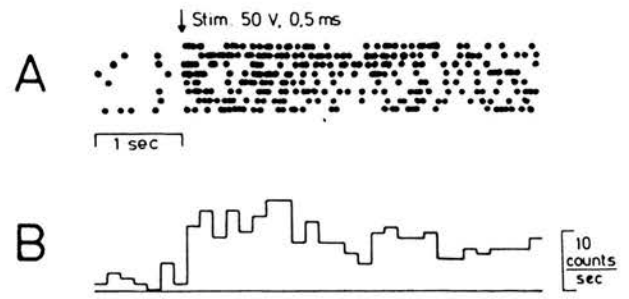

Fig. 4. A: dot display of the discharge pattern of a midbrain unit during electrical single shock stimulation above C-fibre threshold. The stimulus was applied 1/10 sec at the time indicated by the arrow. First stimulus on the top line. B: peristimulus time histogram of the same discharges.

seen in the spinal cord following noxious stimulation, sometimes even in units lacking a strictly temporally locked, distinct excitation by the stimulus. However, "wind up" phenomena were less frequently encountered than the oscillations described above. An oscillating firing pattern has been described previously in midbrain neurones of the rat following noxious stimulation [18].

(3) Responses to natural stimulation of the skin

As stated above, 75 units could be driven by "natural" skin stimulation. Only one of these units was driven exclusively by radiant heat stimulation, the others were activated by mechanical stimuli or by both types of stimulation. All units were tested carefully over the whole posterior and on the scrotum with the different kinds of mechanical stimuli described in Methods. The belly was inaccessible for stimulation because of the position of the animal in the stereotactic frame. The hindpaws and the tail were also tested with radiant heat at different sites. Fifty-nine units, i.e., about 80% of the mechanosensitive units, responded phasically to slight tapping of the skin, the receptive fields not being smaller than one body quadrant. Fifteen units out of this sample were driven in addition by stroking the fur of the rat with a brush, presumably responding therefore to input from hair follicle receptors. Twenty units responding phasically to tapping also exhibited a tonic high frequency response to strong squeezing of skin folds. The latter stimulus was certainly noxious. Sometimes the receptive field for the squeezing response seemed to be wider than that for the tapping response. Receptive field sizes were not studied systematically, however. Eleven units did not respond to slight mechanical stimulation, but only to squeezing, or to squeezing and to noxious heat. Noxious radiant heat served as a quantitatively adjustable stimulus in this study. Two programs were used, one which raised the skin surface temperature stepwise (cf., ref. 14) up to noxious levels, the other which raised it in a ramp function. These two forms of radiant heat stimulation were used to get a rapid account of the relationship between firing frequency and the stimulus parameters "level of temperature" and "rate of change of temperature", since a more thorough and lengthy study of the quantitative relationship using single heat pulses would have interfered with the other aims of this study. As can be seen from Fig. 5, firing of midbrain units usually did not closely follow the form of the stimulus.

Regardless of the slope of the stimulus temperature most units showed a more or less pronounced "switching" to higher discharge frequencies when noxious levels were reached. At noxious levels, however, the firing frequency usually did not appear to closely reflect the level of temperature, as can be seen from the histograms in Figs. 5 and 6. An analysis of interspike interval distributions at different temperature levels (not documented in this paper) supported this conclusion.

Often (cf., Fig. 5), but not always (cf., Fig. 6) an excitation by noxious radiant heat was followed by long lasting afterdischarges, sometimes oscillatory in character. Oscillations of discharge frequency after heat stimulation

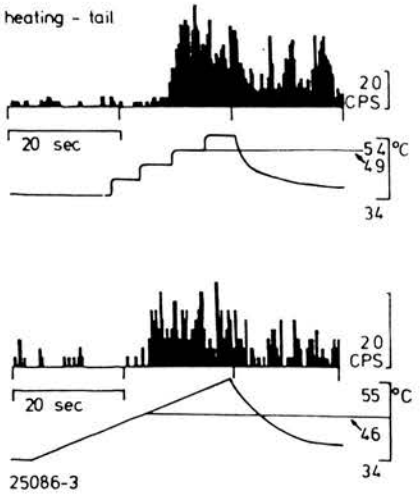

Fig. 5. Spike occurrence density histograms of a unit responding to noxious heating of a spot in the middle of the tail. The skin temperature was raised in a staircase function in the upper record, and in a ramp function in the lower record. The two stimuli were applied at 3 min intervals. Each histogram constructed from a single stimulus.

and oscillating discharges after electrical C-fibre stimulation were not always seen in the same units. Usually a unit, which could be excited by heating of the tail, could also be driven from the hindpaws (cf., Fig. 6). Commonly, however, the receptive field had a centre of greatest input, extending over tail base and left hindpaw in the case of the unit demonstrated in Fig. 6.

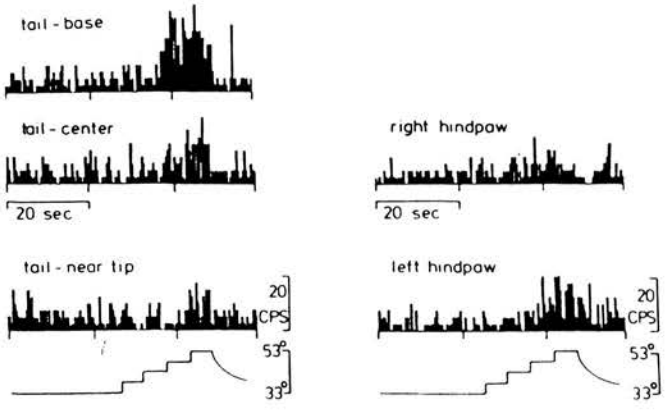

Fig. 6. Spike occurrence density histograms of a single unit on heating different skin sites. The heating program raised the skin temperature in a staircase function, as shown in the traces at bottom. Each histogram constructed from a single stimulus.

TABLE I

		Units responding to heating and/or squeezing		
		+	—	Total
Units responding to electrical	+	39	5	44
C-fibre stimulation	—	6	25	31
Total		45	30	75

(4) Correlation between different types of input

Although the receptor spectrum in the rat's tail is not well known, it can be concluded from our knowledge of cutaneous receptors in other mammalian species that heavy squeezing with serrated forceps and heating above 45°C excite C-fibre nociceptors. Both types of noxious stimulus might excite either the same population of polymodal nociceptors [5], or partly overlapping populations of heat- and mechanonociceptors [3,17].

To test the hypothesis that C-fibre input is relevant for responses to noxious stimulation, we compared the occurrence of C-fibre responses to electrical nerve stimulation of the units responding to heating and/or squeezing the skin with that of units receiving only tactile input. The results are shown in Table I.

Eighty-seven percent of the units responding to noxious skin stimulation, but only 17% of the units receiving a tactile input, exclusively had, in addition, a late response to electrical nerve stimulation above C-fibre threshold. Thus the correlation of a response to "natural" noxious input and a response to electrical C-fibre stimulation is evident and statistically significant ($P < 0.01$, χ^2 test).

In the 6 units receiving noxious input, but showing no C-fibre response, the latter presumably was concealed by a long lasting inhibition following the early A-fibre response of the unit (cf., Figs. 2 and 3).

Thus our results indicate that responses to noxious radiant heat and to noxious squeezing are most likely mediated by C-fibre input. Table II shows the relationship between the two types of "natural" noxious input.

Fifty-one percent of all units responding to any type of noxious stimulation responded to both. The units responding to heating and also to squeezing represent 31% of the sample of units which were found to be responsive

TABLE II

		Units responding to heating		
		+	—	Total
Units responding to squeezing	+	23	8	31
	—	14	30	44
Total		37	38	75

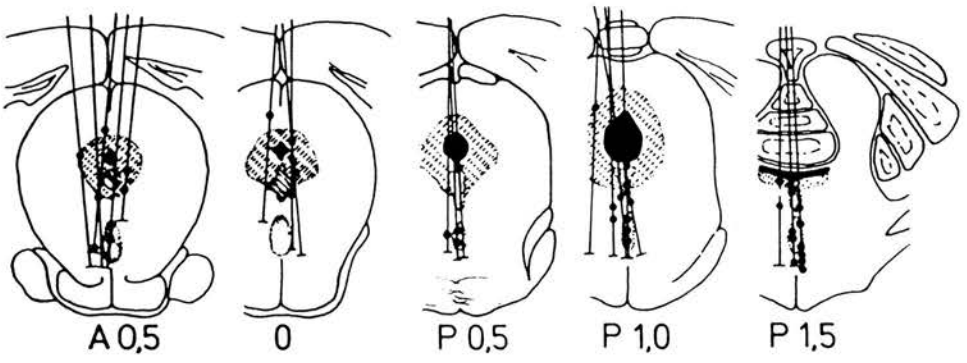

Fig. 7. Histological reconstruction of electrode tracks according to the atlas of Koenig and Klippel, and the supplementary figures of Palkovits and Jacobowitz. Only tracks in which units responding to noxious heating and/or squeezing were recorded are shown. These units are indicated by dots. The horizontal strokes at the bottom of each track indicate the location of the electrolytic lesion. Area covered by a raster: central grey. Hatched areas: raphe nuclei.

to any kind of "natural" cutaneous input whereas another group of units, 40% of the sample, received neither form of noxious input, only tactile input. Again the correlation is statistically significant ($P < 0.05$, χ^2 test). One possible reason for the latter correlation is that polymodal nociceptors are responsible for both heating and squeezing responses. A convergent input from heat- and mechanonociceptors to the same units cannot be excluded, however.

(5) Location of units responding to noxious stimulation

Fig. 7 shows the locations of units responding to noxious input. No functional differences were found between the nociceptive neurones found in different structures of pons and midbrain. It can be seen that those units were found in the central grey, in the mesencephalic and pontine reticular formation, and in different raphe nuclei: the n. medianus raphe, the n. dorsalis raphe and the n. raphe pontis. Location in raphe nuclei does not necessarily imply that serotonergic neurones found in these raphe nuclei are included in our sample, since only some of the units in the raphe region are serotonergic [33]. More units have been found caudal than rostral to the stereotactic zero plane. More input from the tail and the hindpaws to caudal than to rostral midbrain regions has already been suggested previously [12, 20,35].

DISCUSSION

In this study the electrical activity of neurones was recorded in the central grey, in the region of raphe nuclei and in the pontine and mesencephalic reticular formation. Their response patterns to somatosensory input had

some similarity to those described previously in reticular fields [1,2,4,6,9,10, 12,31,36] and in raphe nuclei [18,28] of the midbrain and lower brain stem of the cat. Characteristics of the latter are wide receptive fields, a low degree of somatotopic organization and sensitivity to different qualities of cutaneous input.

Most of the units of our sample could be excited by slight mechanical stimuli and responded with a sustained discharge of higher frequency to noxious squeezing and/or heating of the skin. Sixteen percent of the sample of units which could be excited by natural skin stimulation responded only to noxious levels of stimulation. One could regard these units as "specific nociceptive" neurones. There are, however, arguments against this classification: all units of this sample responding only to noxious natural stimulation also responded to low level electrical stimulation which excited only the A-fibres in the coccygeal nerve. Some of them had very low electrical thresholds and thus certainly received input from thick myelinated nerve fibres. As far as we know, mammals have nociceptors only among the thin myelinated and unmyelinated fibre classes [3,5,7,17]. It is likely therefore that this input from myelinated fibres is a mechanoceptive one. It might have been too weak under the conditions of our preparation to elicit spikes during natural stimulation.

In the present sample of brain stem neurones in the rat, we have found in addition to the above features that the discharge frequency did not appear to reflect closely the level and the rate of change of noxious heat stimuli delivered in the form of ramps or staircases. A previous study with similar stimuli showed a much closer relationship between these stimulus parameters and the firing frequency of nociceptive C-fibres in the cat's plantar nerves [14].

Another feature was the long afterdischarges which often followed noxious heating and squeezing. In particular we did not find qualitative differences of nociceptive responses of units located either in the reticular formation, in the central grey, or in the raphe nuclei. The units responding to noxious cutaneous inputs seem to be scattered throughout these areas.

The functional role of the nociceptive units in pons and midbrain is still not clear. They may be part of the ascending reticular activating system. A second hypothesis is that these units are part of a central pain processing system. Ascending nociceptive pathways passing not only through the mesencephalic reticular formation but also through the central grey have been postulated on the basis of behavioural deficits of cats with lesions in this brain region [27]. The fact that electrical stimulation of the medial midbrain often evokes escape reactions in animals [16] and the sensation of pain in man [29] supports such a hypothesis. Even some of the experiments in which it was shown that pain reactions can be suppressed by electrical stimulation of the central grey [22—24] or by local action of morphine [11,15, 21,35] perhaps fit into the hypothesis of an ascending pathway in this brain region. Morphine might probably suppress units of such a pathway and if high currents (more than 1 mA) are used in experiments with electrical

brain stimulation, these could induce inexcitability in some neurones for some time.

However, if the "pain suppression centre", which has been presumed to be situated in this region, exists, and if units receiving nociceptive input are involved in its action, our findings are consistent with the concept of a negative feedback loop which is effective in suppressing pain.

We have shown in this study that the nociceptive input reaching neurones in pons and midbrain of the rat is most probably transmitted by C-fibres in the coccygeal nerves. Response patterns to electrically evoked C-fibre volleys have rarely been described previously in brain stem neurones. Two studies dealing with these responses in the cat reported extremely long latencies [4, 10] requiring a central conduction time of at least 1 sec in one case [4]. C-fibre responses with shorter latencies, indicating an oligosynaptic input, have been reported in the monkey midbrain [25]. The present findings in the rat suggest the existence of oligosynaptic input from C-fibres to midbrain neurones in this species. One cannot exclude, however, a possible contribution from slowly conducting Aδ-fibres, which were either too few in number or which had such different conduction velocities that they were not visible in the compound nerve action potential.

Yet all units (even the sample of 18% units which were driven by nociceptor input, but could not be excited by non-noxious tactile input) could also be activated by A-fibre stimulation in the nerve. Because of this 2-fold electrical input we do not assume that any of them are unimodal units, e.g. exclusively nociceptive in the sense that they are anatomically connected only to nociceptors. It could be, of course, that part of the input is suppressed in some behavioural situations and thus units are functionally unimodal.

Any type of anaesthesia or decerebration induces a rather extreme functional state which might influence the properties of central neurones. Chloralose as used in this study has been found previously to enhance the receptive field to tactile input [1]. Responses to noxious stimulation might be less frequent than in the decerebrate state [8]. Nevertheless, we have found a high proportion of units responding to noxious input in our study. This may indicate the functional importance of nociceptive input to the medial structures of the upper brain stem in the rat.

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1 PERIPHERAL ARTERIAL CHEMORECEPTORS

D.S. McQueen and D.J. Pallot

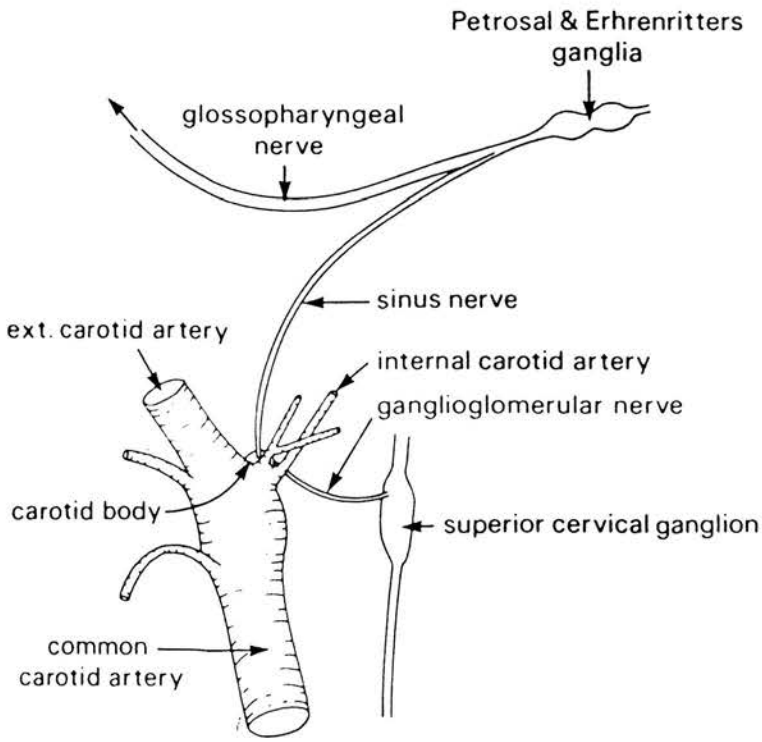
This chapter describes the influence of the peripheral arterial chemoreceptors on respiration and also examines the transduction mechanism of these receptors. It is our intention to concentrate mainly on the recent literature; readers requiring information about historical aspects, or more detailed evidence than it is possible to give in this chapter, are referred to reviews on chemoreceptors (e.g. Schmidt & Comroe, 1940; Heymans & Neil, 1958; Dejours, 1962; Anichkov & Belen'kii, 1963; Torrance, 1968; Biscoe, 1971; Eyzaguirre & Fidone, 1980) and the proceedings of recent international meetings on arterial chemoreceptors (Purves, 1975; Paintal, 1976; Acker *et al.*, 1977; Belmonte *et al.*, 1981).

The peripheral arterial chemoreceptors are sensory receptors which monitor the oxygen and carbon dioxide tensions and pH of arterial blood, and form part of the integrated system that controls respiration. A fall in the partial pressure of oxygen in arterial blood (PaO_2), a rise in $PaCO_2$ or a fall in pH, increases the activity of these receptors, increases discharge frequency in their sensory (afferent) nerves, which terminate centrally mainly in the nucleus tractus solitarius, and ultimately may stimulate respiration. Two main groups of arterial chemoreceptors exist in mammals, namely the aortic and carotid bodies located, respectively, near the arch of the aorta and in the bifurcation of the common carotid arteries. The carotid body is very vascular and is supplied with arterial blood by one or more arteries originating from the bifurcation or the external carotid artery; venous blood drains into the internal jugular vein. The aortic bodies are supplied with blood from small branches of the aortic arch. In addition, pulmonary chemoreceptors have been described (Coleridge, Coleridge & Howe, 1967) and tissue similar in structure to that of chemoreceptors is found along the course of the abdominal aorta (abdominal paraganglia, Abbot & Howe, 1972). This review will only be concerned with chemoreceptors of the aortic and carotid bodies because it is these which have been most extensively studied and they appear to be of greatest importance.

Each carotid and aortic body receives nerve fibres from two sources. The glossopharyngeal (cranial IX) nerve supplies sensory fibres to the

carotid body (glomus caroticum) via a branch known variously as the carotid nerve, the intercarotid nerve, the carotid sinus nerve, the sinus nerve or Hering's nerve, and the cell bodies of these sensory fibres are located in the sensory (petrosal) ganglion of the IXth nerve. In addition, the carotid body is also innervated by sympathetic fibres (ganglio-glomerular nerves) from the superior cervical ganglion (see Figure 1.1). Some sympathetic fibres also course down the sinus nerve. The aortic bodies are innervated by the (aortic) vasodepressor nerve (a branch of the vagus) which has its cell bodies in the nodose ganglion; they also receive a sympathetic nerve supply from the stellate ganglion.

Figure 1.1: A Diagrammatic Representation of the Innervation of the Cat Carotid Body. Note the dual innervation from the sinus nerve and ganglioglomerular nerve. The aortic bodies also receive a dual innervation.



Histologically the carotid and aortic bodies consist of groups of cells collected around numerous large capillaries. A connective tissue stroma is found between groups of cells and contains numerous nerve fibres; on closer examination each cell group is seen to contain two cell types which have been given a variety of names (see Biscoe, 1971). We shall apply the term Type I to the most common type of cell, and Type II for the less frequently encountered cell which is often located at the periphery of a cell group. The general histological features of the carotid body are illustrated in Figure 1.2; and we shall make a more detailed examination of the important structural features of peripheral chemoreceptors in a later section.

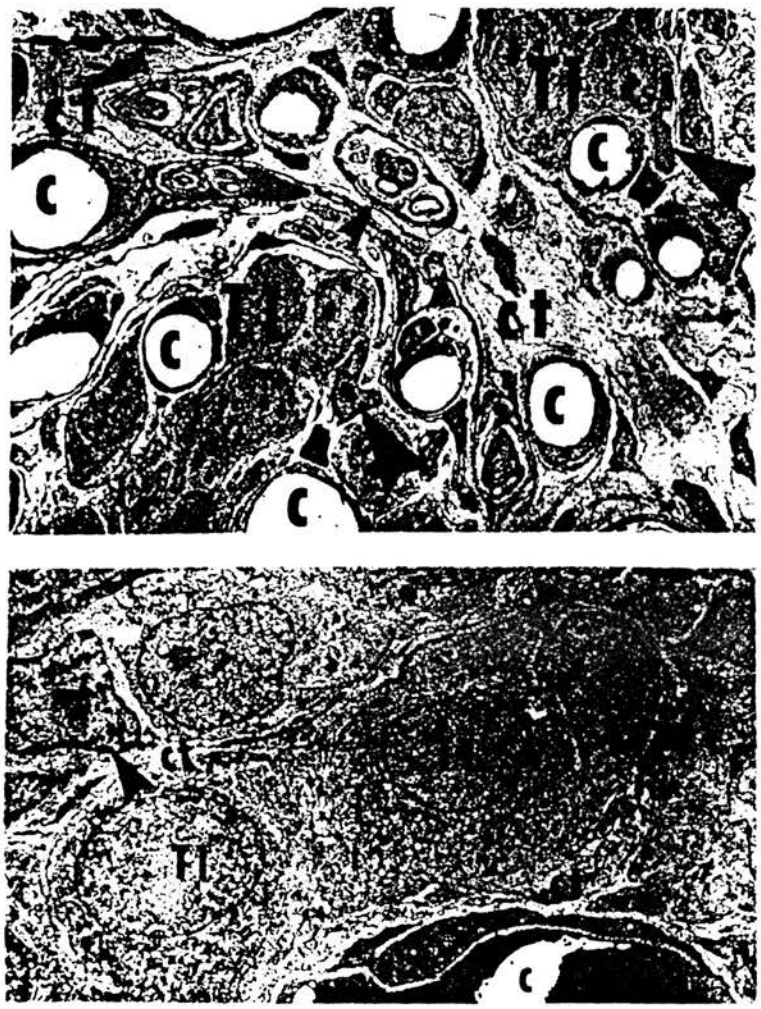
The embryological origin of the carotid body has only recently been established. Using a heterospecific grafting technique (Le Dovarin, Le Lievre & Fontaine, referred to by Verna, 1979) which enabled donor cells to be identified in the recipient, Pearse and his colleagues (see Verna, 1979) established that in birds Type I cells were definitely derived from neural crest tissue, and Type II cells may also originate from the same source. A number of studies have described the development of the carotid body (see Verna, 1979); all of these demonstrate that the carotid body is structurally mature well before birth, a somewhat surprising finding in view of the physiological immaturity of the organ prior to the onset of respiration (see Chapter 8).

Two particularly interesting features of the carotid chemoreceptors emerged from studies performed on cats. They have a very high oxygen consumption of $\simeq$ 9ml $(100g^{-1})min^{-1}$, indicative of intense metabolic activity, together with a very substantial blood flow of 2 litres $(100g^{-1})$ min^{-1}. The difference between arterial and venous blood oxygen content is, however, fairly low (0.2 - 0.5 ml/100ml) and the venous blood is quite red; this could result from low oxygen extraction from the blood, or from direct passage of blood from arteries to veins via extensive anastamoses in the carotid body.

Study of Peripheral Arterial Chemoreceptor Activity

Various techniques are employed for studying chemoreceptor activity, including the classical approach of using reflexly induced respiratory changes as an indicator of receptor activity (see Heymans & Neil, 1958). Electrophysiological techniques are now commonly used because they avoid some of the complications associated with reflex studies, such as the influence of the anaesthetic on the reflex pathway, or

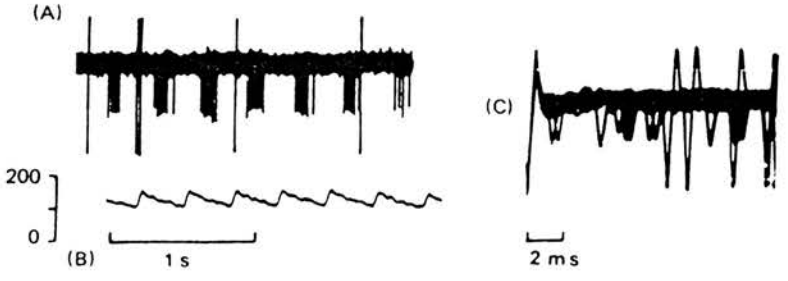
Figure 1.2: The Histology of the Carotid Body. Note how the cells are set in a connective tissue stroma (ct) amidst numerous blood vessels (c). Some bundles of nerve fibres are also seen (small arrow head). Even at this magnification two cell types are visible on the basis of nuclear morphology (T1 and large arrow heads), and position in the cell groups.



secondary changes such as those initiated by alterations in blood gas tensions or by the lung inflation reflex. Such techniques are generally regarded as providing more information about activity at the receptor; what they actually provide is information from a small sample of chemosensory fibres which is taken to represent the response of the whole carotid body complex.

Recordings of single units (single active fibres) obtained using extracellular recording electrodes show that action potentials associated with chemoreceptor fibres occur irregularly. This distinguishes them from the regular pressure-related discharge associated with baroreceptor fibres (Figure 1.3). The interval between successive chemoreceptor potentials is in fact random, following a Poisson distribution (see Biscoe, 1971). Furthermore, unlike the baroreceptors which cease firing when the arterial blood pressure falls below a certain level, chemoreceptor afferents do not exhibit a firing threshold.

Figure 1.3: Electrical Recording of Sensory Activity in a Filament Dissected from the Peripheral End of a Carotid Sinus Nerve which had been Cut Centrally (A). Note the irregular discharge of the large biphasic chemoreceptor unit and the regular discharge of the smaller monophasic baroreceptor unit which is synchronous with the pulse seen in the blood pressure record (B) (McQueen, unpublished record). In (C) the lower part of the chemoreceptor action potential was used to trigger 50 successive sweeps of a storage oscilloscope during intense chemoreceptor stimulation evoked by sodium cyanide (5 μg intra-carotid); it can be seen from the trace on the right-hand side of the figure that no chemoreceptor action potential occurred within 7 ms of a triggering potential. This observation, together with the shape and amplitude of the action potential, enables it to be classified as a single unit.



The effects on chemoreceptor discharge and on respiration of breathing gas mixtures containing different concentrations of CO_2 and O_2 have been studied by various workers using both respiratory and electrophysiological techniques (e.g. Lahiri & Delaney, 1975).

Function

As already mentioned, the arterial chemoreceptors respond to changes in arterial blood gas tensions and pH and reflexly influence respiration. The chemoreceptors are responsible for about 15 per cent of the respiratory drive during eupnoeic (normal) respiration, most of this contribution appears to come from the carotid chemoreceptors in man. The carotid body is the only mechanism in the body for detecting hypoxia and initiating rapid reflex respiratory adjustment. The central chemoreceptors are not stimulated by hypoxia; rather breathing is depressed by the direct effect of hypoxia on the CNS. This effect is in contrast to that of CO_2-H^+ in the physiological range which can act centrally to increase respiration, as well as reflexly via the arterial chemoreceptors. Very high levels of CO_2-H^+ can cause a central depression of ventilation. In this chapter we are concerned exclusively with the influence of arterial chemoreceptors on respiration, but it should be appreciated that these receptors reflexly influence other body functions, such as control of the cardiovascular system (Heymans & Neil, 1958; Anichkov & Belen'kii, 1963).

Peripheral chemoreceptor discharge increases when either PaO_2 falls or the $PaCO_2$ rises. The two stimuli interact in the peripheral chemoreceptors in such a way that hypoxia enhances the discharge associated with hypercapnia, and vice versa. The interaction is additive or more than additive (multiplicative) at lower discharge frequencies. Clearly as the response to either stimulus alone approaches maximum, the potentiation produced by the two stimuli given together is less marked. There is not much change in ventilation when PaO_2 falls during normocapnia in steady-state conditions, until a PaO_2 of about 8.7 kPa (1 kPa $\simeq$ 7.5 mmHg) is reached when breathing increases. Peripheral chemoreceptor activity, however, increases when slight falls in oxygen tension occur even at levels of PaO_2 well in excess of the respiratory 'threshold'; chemoreceptor activity is not linearly related to PaO_2, the curve of discharge against PaO_2 steepens as the PaO_2 falls. The explanation for the lack of correlation between chemoreceptor discharge and ventilation is that the peripheral chemoreceptor drive is

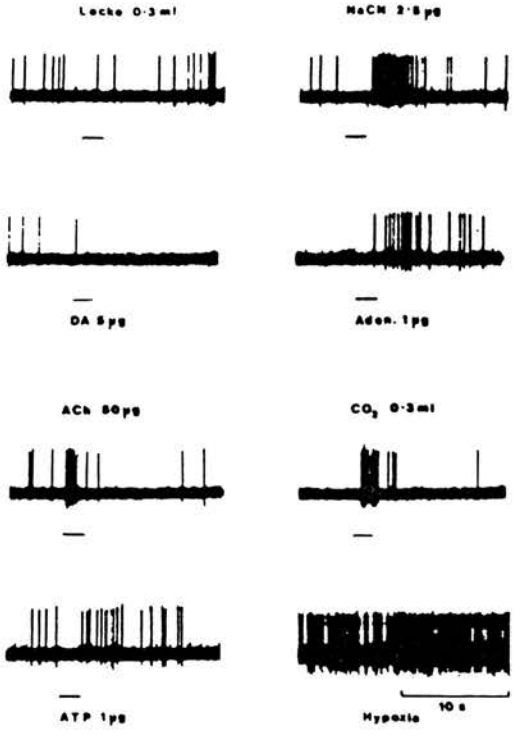
opposed by direct depression of the respiratory centre by hypoxia; additionally the fall in $PaCO_2$ that occurs in response to the hyperventilation decreases the drive from the central chemoreceptors. When a critical PaO_2 is reached, these factors are unable to negate the increased input from the peripheral chemoreceptors, and respiration increases.

There is an almost linear relationship between carotid chemoreceptor discharge and $PaCO_2$ over the physiological range, the slope of the curve steepening as PaO_2 is lowered. A 'fan' of lines relates respiration to $PaCO_2$ at different oxygen tensions in man (see Cunningham, 1974). Below a certain $PaCO_2$, known as the 'CO_2 threshold', ventilation appears to be independent of $PaCO_2$ (even though peripheral chemoreceptor activity is not) and the overall CO_2-ventilation curve is often described as being 'dog-legged' in shape, something which is most apparent under hypoxic conditions. Experiments in anaesthetised animals show that ventilation is not totally independent of $PaCO_2$ in the range 2.7 - 5.3 kPa, and apnoea can occur even in severe hypoxia when the $PaCO_2$ is very low. However, anaesthesia interferes with the responses, as is shown by the fact that anaesthesia in man raises the CO_2 threshold and the 'dog-leg' is not seen. Respiratory thresholds vary from individual to individual, and even within individuals; it is thus very difficult to obtain meaningful data from conscious animals and man when breathing is subject to minimal drive because ventilation becomes very irregular (see Cunningham, 1974). Hence it is difficult to be sure what the CO_2-respiration dog-leg signifies, but it may result from CO_2-H^+ acting in the medulla to modify afferent input from the peripheral chemoreceptors.

Experiments in man using the single breath O_2 test (see Dejours, 1962) enable assessment of peripheral chemoreceptor respiratory drive to be made since the single breath will influence the arterial chemoreceptors before the CNS, and changes in respiration will not be complicated by the alterations in blood gas tensions and pH which accompany alterations in ventilation under steady-state conditions. It is found that a single breath of 100 per cent O_2 causes a transient fall of about 15 per cent in respiration. This response has a latency of 1-3 breaths due mainly to the circulation time from alveoli to arterial chemoreceptors, and is not obtained when the peripheral arterial chemoreceptors are denervated. If oxygen breathing continues, respiration returns to normal because although removal of peripheral chemoreceptor drive initially reduces ventilation, CO_2 retention occurs and the rise in $PaCO_2$ stimulates respiration, so that within one minute

breathing is back to normal. This can be seen in Figure 1.5, where respiration during breathing 100 per cent O_2 in steady state is not much different from that during air-breathing, although carotid chemoreceptor discharge continues to be reduced by the high oxygen tension.

Figure 1.4: Discharge of a Single Chemoreceptor Unit Showing the Effects of Intracarotid Injections of Different Substances at the Marker. Also shown is the maximal discharge recorded 90 s after starting to ventilate the animal with 100 per cent N_2 instead of air. (McQueen, unpublished cat data.)



Peripheral chemoreceptors can respond to CO_2 or H^+, but the response to H^+ is not as fast or intense as that to a transient CO_2 stimulus producing the same pH change. This is probably because molecular CO_2 crosses biological membranes faster than H^+. Carbonic anhydrase inhibitors such as acetazolamide slow the hydration of CO_2

and reduce the speed and intensity of the chemoreceptor response to CO_2, which suggests that at least part of this response involves conversion of CO_2 into H^+/HCO_3^-. Part of the effect has been attributed to molecular CO_2 because the chemoreceptor response to CO_2 under steady conditions is greater than that to the equivalent change in H^+. The precise stimulus (CO_2 and/or H^+-HCO_3^-) at the peripheral chemoreceptor remains to be determined, and it will be necessary to ensure that drugs like acetazolamide act specifically as inhibitors of carbonic anhydrase in the chemoreceptors, and do not have other actions which affect the response of these receptors to CO_2.

There has been some controversy concerning the ability of CO_2-H^+ to stimulate the aortic chemoreceptors, and Hanson, Rao & Torrance (1979) who investigated the CO_2 sensitivity of the cat aortic chemoreceptors concluded that although some of the aortic chemoreceptors do respond to CO_2, the responses are very variable in the steady state. This is in contrast to the marked effect CO_2 has on the carotid chemoreceptors, and the authors consider that the difference may be due to a greater adaptation of the aortic receptors to CO_2. The fact that single carotid chemoreceptor units can respond to both CO_2 and hypoxia, as well as other stimuli, is illustrated in Figure 1.4.

It is generally agreed that activation of the carotid chemoreceptors increases the tidal volume and, to a lesser extent, the frequency of respiration. Aortic chemoreceptor stimulation has less effect on tidal volume, but does increase respiratory frequency. The overall increase in respiratory minute volume in response to the same stimulus is about seven times greater for the carotid chemoreceptors than it is for the aortic chemoreceptors in anaesthetised dogs (Daly & Ungar, 1966). However, the overall contribution to respiration made by the aortic and carotid bodies varies from species to species and depends on the techniques used. Humans with denervated carotid bodies but intact aortic bodies do not show an increase in tidal volume when exposed to hypoxia whereas those with intact carotid bodies do (Wasserman & Whipp, in Paintal, 1976). Hypoxia depresses the CNS and might mask the input from aortic chemoreceptors, but people who had undergone bilateral carotid body resection and were not hypoxic showed no significant increase in ventilation following i.v. injection of doxapram, a drug which can stimulate the peripheral chemoreceptors, whereas normal individuals responded to the same dose with a significant increase in ventilation (Honda *et al.*, 1979). Smith & Mills (1980) however have demonstrated that cats which have undergone bilateral section of the sinus nerve develop a renewed sensitivity to hypoxia

after 2–3 months, presumably due to stimulation of the aortic bodies.

Chemoreceptor stimulation also indirectly affects respiration by reflexly affecting bronchial airflow. Nadel & Widdicombe (1962) concluded that carotid chemoreceptor stimulation in dogs reflexly constricts the upper and lower airways, thus leading to changes in blood gas tensions. Reflex changes in cardiac output and catecholamine secretion from the adrenal gland may also indirectly affect blood gas tensions. There is evidence that sudden application of stimuli (i.e. abrupt changes in blood gas tensions) evoke respiratory responses only if they reach the peripheral chemoreceptors at the appropriate point of the respiratory cycle. Thus, chemoreceptor stimulation during inspiration leads to an augmented breath and an increase in respiration, whereas the same stimulus arriving during expiration may have no effect on respiration (Black & Torrance, 1967). This type of stimulus is not very physiological, but oscillations of PO_2, PCO_2 and pH related to respiratory periodicity do occur in arterial blood (see Chapter 7).

Cat carotid chemoreceptors can follow oscillations up to a frequency of about 20 Hz for PaO_2 and 70 Hz for $PaCO_2$, the faster the frequency, the smaller the oscillation in chemoreceptor discharge. The receptors are, therefore, capable of signalling breath to breath changes in blood gas tensions and pH. The phase relationship between chemoreceptor afferent discharge and activity of the inspiratory neurones may well be important in adjusting respiration during speaking, eating, swallowing, yawning, laughing, crying and, perhaps, exercise. Temporal differences between aortic and carotid chemoreceptor input to the CNS could theoretically be used to compute blood/gas flow to the brain, but recent work by Lahiri *et al.* (1980a) revealed that the latency to onset of response following changes in the gas mixture breathed, or intravenous injection of chemoreceptor stimulants such as cyanide, is greater for the aortic than it is for the carotid chemoreceptors in cats. Whether the CNS makes use of the temporal difference between carotid and aortic chemoreceptor input, or input from myelinated and unmyelinated fibres, remains to be established. It has also been found that rapid adaptation of chemoreceptor discharge occurs when CO_2 is used as a stimulus, but not when hypoxia is used (Black, McCloskey & Torrance, 1971) and the response to CO_2 is generally more rapid in onset than that to hypoxia. The rapid or dynamic nature of the response to peripheral chemoreceptor stimulation is a particularly marked feature of these receptors and is illustrated in Figure 1.4.

Hypoxia can be induced in tissues receiving a normal blood flow by lowering the oxygen tension of the blood (i.e. hypoxic hypoxia affecting

the amount of oxygen dissolved in the plasma) or, by causing histotoxic hypoxia, as does cyanide, by inhibiting cytochrome oxidase. Examples of both these methods have already been given. An additional method of producing hypoxia is to reduce the amount of oxyhaemoglobin in the blood, leading to anaemic hypoxia, and this can be achieved by lowering the number of red blood cells or by using carbon monoxide to form carboxyhaemoglobin, thereby reducing the oxygen content of the blood without affecting the oxygen tension. One of the reasons why carbon monoxide is so dangerous is that no hyperventilation occurs during poisoning with this gas. There has been considerable controversy over the question of whether or not carbon monoxide stimulates arterial chemoreceptors, some workers finding carbon monoxide to be without effect on carotid chemoreceptors, others that it stimulates the aortic chemoreceptors and/or the carotid chemoreceptors. Recent work by Lahiri *et al.* (1981) appears to clarify matters by showing that in cats in which carotid and aortic chemoreceptor discharge are recorded simultaneously, carbon monoxide inhalation during normoxia always stimulated aortic chemoreceptors before it affected the carotid receptors, and that the steady-state response of aortic chemoreceptors to carboxyhaemoglobin was greater than that of most carotid chemoreceptors; only about 10 per cent of the carotid chemoreceptor fibres tested showed any increase in discharge frequency in response to moderate increases in carboxyhaemoglobin. The authors hypothesise that the aortic bodies have a much lower perfusion relative to their oxygen utilisation compared to the carotid bodies, and as a consequence are able to monitor oxygen delivery and initiate circulatory reflexes for oxygen homeostasis; whilst carotid chemoreceptors monitor oxygen tension and initiate strong reflex effects on respiration.

Thus, the carotid body chemoreceptors seem to be satisfied by the oxygen dissolved in the plasma, and carbon monoxide poisoning is unlikely to cause any reflex respiratory changes via the peripheral chemoreceptors until the levels of carboxyhaemoglobin are quite high. Results obtained from experiments in which the haematocrit was reduced are consistent with the carbon monoxide results, a decrease in the number of red blood cells during normoxia stimulated the aortic chemoreceptors, but much larger reductions in haematocrit seldom affected the carotid chemoreceptors provided the PaO_2 was above 8.0 kPa.

Blood Flow

The carotid body blood flow rate is very high, as already mentioned. Purves (1970) performed experiments in anaesthetised cats from which he concluded that carotid blood flow of the intact innervated carotid body is linearly related to mean arterial pressure over the range 13.3 - 22.7 kPa, and hypoxia or hypercapnia can cause a small increase in carotid body blood flow. Others have presented evidence that autoregulation of blood flow occurs in the carotid bodies of some species. In many vascular beds trauma, or extremes of perfusion pressure, abolishes autoregulation, and great care has to be taken not to damage what can be a fairly delicate mechanism. Changes in arterial blood pressure within the physiological range do not cause any significant alteration in carotid chemoreceptor discharge, but do affect the aortic chemoreceptors; a fall in pressure increases discharge. It is interesting to note that when a cat carotid body is removed from the animal and superfused *in vitro*, chemoreceptor discharge recorded from the carotid sinus nerve increases rapidly in response to a reduction in flow of the superfusing fluid. Oxygen usage is also reduced. The flow-sensitivity of the *in vitro* carotid body thus makes it more akin to the *in vivo* aortic body.

The capillaries of the carotid body are fenestrated, and numerous A-V shunts exist. There has been much discussion and experimental work concerning the distribution of blood within the carotid and aortic bodies and the suggestion has been made that 'plasma skimming' may occur. This arises from non-Newtonian fluid flow in narrow tubes, and the suggestion is that as the speed of the current is greatest and the pressure lowest at the centre of the blood vessel, red cells will migrate to the central axis and travel faster than the plasma at the side walls. A small branch from a larger artery would receive blood with a lower proportion of red cells, i.e. the plasma has been skimmed. Such a mechanism would explain the observations by Acker & Lübbers (in Acker *et al.*, 1977) that the decrease in tissue PO_2 is the same under zero flow conditions whether the organ is perfused by blood or saline equilibrated with air. These authors propose that during progressively increasing hypoxia an increasing number of red cells enter the carotid body. The idea is attractive for it enables the tissue PO_2 to be maintained. Unfortunately, however, preliminary quantitative histological studies on the area occupied by red cells within the carotid body vasculature at different levels of PaO_2 in rabbits do not support this concept (Pallot & Verna, unpublished observations).

Experiments with oxygen-sensitive microelectrodes (e.g. Acker & Lübbers in Acker *et al.*, 1977) have established that the PO_2 in carotid body tissue is about 5.3 kPa, much lower than the PaO_2. However, differing results have been obtained concerning the oxygen gradient within the carotid body, and further refinement of the technique will be needed before one can obtain reliable information concerning the oxygen tension in the vicinity of the nerve endings and in the cells.

Influence of Exercise on Chemoreceptor Discharge

There has been considerable debate concerning the question of whether or not arterial chemoreceptors contribute to the hyperpnoea of exercise (see Cunningham (1974) for a detailed discussion of breathing in exercise). There is some evidence which favours peripheral chemoreceptor involvement. Thus, administration of oxygen reduces the hyperpnoea of exercise, the respiratory response to exercise in ordinary individuals is potentiated by hypoxia and it is absent in people who have undergone bilateral carotid resection, as is their late, but not early, respiratory response to exercise (Wasserman & Whipp, in Paintal 1976). If arterial chemoreceptors are involved in exercise hyperpnoea, what is it that activates the receptors? PaO_2, $PaCO_2$, pH and body temperature change only slightly in the early stages, and although the possibility exists that the sensitivity of the peripheral chemoreceptors to these variables may alter during hypoxia, other influences need to be considered. Sympathetic activity increases during exercise and increased sympathetic drive to the peripheral chemoreceptors can increase ventilation. Some results in man accord with this idea, but Eisele, Ritchie & Severinghaus (1967) concluded that the steady-state ventilatory response to moderate exercise in man is not influenced by sympathetic innervation of the chemoreceptors since blocking the stellate ganglia had no marked effect on the hyperpnoea of exercise. Experiments in anaesthetised cats showed that moving the hind limbs caused an immediate increase in carotid chemoreceptor discharge which was abolished by removing the sympathetic innervation of the carotid body or cutting the femoral and sciatic nerves (see Biscoe, 1971). Could it be that in exercise, increased activity in muscle afferents reflexly increases chemoreceptor discharge? Not according to Davies & Lahiri (1973), who also performed experiments on cats (anaesthetised or decerebrated), since they were unable to show any change in carotid chemoreceptor activity during exercise, although hyperpnoea occurred.

The present position concerning the involvement of peripheral chemoreceptors in exercise hyperpnoea is somewhat confusing, and further studies appear to be necessary in order to establish their role. It could be that muscle afferent activity acts centrally to increase the influence on respiration of a given chemoreceptor input. It is also possible that during exercise, changes in the oscillations of blood gas tensions, cardiac output, circulating catecholamines or other hormones, and carotid sinus nerve efferent activity, might influence arterial chemoreceptor activity by acting either separately or interactively (see also Chapter 7).

Chronic Resection of the Carotid Bodies

Many patients in Japan some 25 years ago underwent bilateral carotid body resection for asthma. This procedure only temporarily relieved their symptoms (see Honda *et al.*, 1979). Treated patients have a normal $PaCO_2$ and pH, but a slightly reduced PaO_2, do not show any reflex increase in respiration while breathing hypoxic gas mixtures in the steady-state; in single breath studies there is evidence of some chemosensitivity. It appears that although aortic chemoreceptors can prevent the central respiratory depression of hypoxia, they do not elicit hyperventilation and so, according to Wasserman & Whipp (in Paintal, 1976), are relatively insensitive respiratory control organs in man. Using few-breath oxygen tests in conscious dogs, Ungar & Bouverot (1980) have demonstrated that even moderate hypoxia can cause a marked depression of respiration. Therefore, it may be that aortic chemoreceptors do make an important contribution to respiration, but this is not apparent during hypoxia in the absence of carotid chemoreceptors because of the substantial depressant effect hypoxia exerts on central respiratory neurones.

Animal experiments show that different species vary in their ability to recover peripheral chemosensitivity following bilateral carotid body resection. Slight sensitivity returns in ponies, but in cats after about 260 days peripheral chemoreflex activity apparently returns to near normal. No chemoreceptor activity was recorded from the regenerated carotid sinus nerve, and bilateral vagotomy abolished the reflex respiratory response to physiological and pharmacological stimuli, implying that the aortic receptors were being stimulated (Smith & Mills, 1980). The suggestion is that, about 30–40 days after bilateral carotid body removal, aortic chemoreceptors increase their influence on

respiration, possibly as a consequence of changes in gain of the central component of the reflex pathway.

High Altitude

This topic will be dealt with in detail in Chapter 9 and we shall confine ourselves to noting here that during adaptation to high altitude, respiration is stimulated by hypoxia acting via the peripheral chemoreceptors; hypocapnia and alkalosis limit the hyperventilation. However, acclimitisation can still occur after chronic denervation of the aortic and carotid chemoreceptors which suggests that although the peripheral chemoreceptors do contribute to the process they are not absolutely essential. It is interesting that carotid bodies taken from high altitude-dwelling man and animals are greatly enlarged. This hypertrophy is also observed in chronically hypoxic individuals at sea level and can be mimicked in the laboratory. Thus, Dhillon, Barer & Walsh (in Pallot, 1983) have shown a sixfold increase in carotid body volume after exposure of rats to 10 per cent O_2 for three weeks. Barer, Chiocchio & Pallot (unpublished) found a massive increase in catecholamine concentration in rat carotid bodies after similar treatment.

Influence of Peripheral Chemoreceptors on Respiration in Neonates

The role of arterial chemoreceptors in initiating respiration, and the influence of sympathetic nerves on peripheral chemoreceptors in the new-born is considered in Chapter 8. We should like to concentrate here on the question of whether failure of peripheral chemoreceptors contributes to the sudden infant death syndrome (SIDS) or 'cot death', in which apparently healthy infants die when aged between about 1 and 10 months (average 2-4 months in the study of Naeye *et al.*, 1976), while asleep. Histological studies of carotid bodies taken from infants who had died of SIDS show abnormalities when compared with carotid bodies taken from age-matched infants dying from other causes, including congenital heart disease (Naeye *et al.*, 1976; Cole *et al.*, 1979).

In general the carotid bodies in SIDS showed a reduction in the size and number of cells and also a decrease in the dense cytoplasmic granules in the cells, something which was not seen in the carotid bodies of infants who had been chronically hypoxic thoughout life

and eventually died of congenital heart disease. This suggests that degranulation in SIDS is not secondary to hypoxia.

A correlation has been suggested between prolonged apnoea during sleep, particularly REM sleep, and SIDS; infants considered at risk for SIDS (Shannon, Kelly & O'Connell, 1977) showed hypoventilation during sleep and a decreased ventilatory response to breathing CO_2. While it is obvious that peripheral chemoreceptor involvement in SIDS is far from established, and other causes have been suggested, there seems sufficient evidence to warrant serious study of the possibility. It could be that in this disorder arterial chemoreceptors do not function normally because of some defect in the receptor mechanism, or alternatively the CNS fails to respond to chemoreceptor input during hypoxia; perhaps excessive efferent activity in the sinus nerve during hypoxia is a contributing factor. It will be necessary to determine how sleep influences the arterial chemoreceptors and respiratory control. There may be a case for using the single breath oxygen test to examine whether peripheral chemoreceptor function is normal in young infants, and this should be done while the child is asleep. Such a test may help to identify those infants who may be at risk, and could give information concerning the role of peripheral chemoreceptors in SIDS which is otherwise going to be difficult to obtain from animal studies.

Peripheral Arterial Chemoreceptors and Respiratory Disorders

Although the arterial chemoreceptors may not be essential for ordinary life in healthy individuals, the situation is very different in unhealthy people. For example, patients with chronic obstructive lung disease (emphysema, chronic bronchitis) are usually hypoxic, hypercapnic and acidotic; such patients have chronically enlarged carotid bodies (see Heath, Smith and Jago in Pallot, 1983).

The consequence of chronic CO_2 retention is that the central chemoreceptors become less responsive to CO_2, respiratory depression occurs, and the balance of respiratory drive shifts from the central to the peripheral chemoreceptors; hypoxaemia depresses the CNS, but the peripheral chemoreceptors are stimulated. A similar situation can arise when the CNS has been depressed by drugs such as barbiturates or opiates.

Injudicious administration of oxygen to raise the PaO_2 in patients such as those described above can, by removing peripheral chemoreceptor drive, stop ventilation, thereby causing a further rise in $PaCO_2$

and a fall in PaO_2. However, there is a definite need to increase the PaO_2 in patients with respiratory depression, particularly when hypoxaemic episodes associated with periods of hypoventilation occur during REM sleep (compare SIDS) in 'blue and bloated' patients who are already hypoxic (Douglas *et al.*, 1979), and this requires controlled oxygen therapy, with or without the assistance of mechanical ventilation or respiratory stimulant drugs.

Drugs which Stimulate the Peripheral Chemoreceptors

Drugs are chemicals and so it is not surprising that a large number of them can affect the arterial chemo (chemical)-receptors. We shall confine ourselves to considering here only those drugs which are fairly specific chemoreceptor stimulants and which may have a clinical use as respiratory stimulants. In passing, however, it is worth noting that one widely used non-specific chemoreceptor stimulant, nicotine, evokes reflex changes in B.P., heart rate, and respiration (see Ginzel, 1975). It remains to be established whether repeatedly stimulating the arterial chemoreceptors by smoking cigarettes causes changes in the receptors, which in turn leads to pathophysiological alterations in the respiratory and cariovascular systems.

Suberyldicholine is a nicotinic receptor agonist which has been advocated for use as a respiratory stimulant (Anichkov & Belen'kii, 1963) as it has advantages over centrally acting drugs. Doxapram appears to act as a peripheral chemoreceptor stimulant (Mitchell & Herbert, 1975) and has been used to test peripheral chemoreceptor functioning in man. Some drugs may appear to be peripheral chemoreceptor stimulants, but in fact act centrally to potentiate input from the chemoreceptors. It is necessary to be cautious when interpreting results from experiments in which respiration is recorded and drug effects studied. Abolition of the respiratory-stimulating property of a drug by peripheral chemoreceptor denervation is not, on its own, sufficient evidence that the drug stimulates the chemoreceptors and more direct evidence, e.g. electrophysiological, is needed. The drug might, for example, have been acting centrally, and changes in the pattern of respiration following denervation may mask this action, or it could have been indirectly affecting ventilation via the baroreceptors since peripheral chemoreceptor denervation generally involves denervating the baroreceptors. Also, evidence from animals, anaesthetised or conscious, is not necessarily directly applicable to man.

A new chemoreceptor stimulant drug, which may be useful in replacing hypoxic drive from the peripheral arterial chemoreceptors during oxygen breathing, is almitrine. Experiments in cats (Figure 1.5) show that intravenous infusion of almitrine increases carotid chemoreceptor discharge and respiration, even when the animal breathes 100 per cent O_2; it does not seem to be a nicotinic agonist since its action was not affected by hexamethonium or mecamylamine. Almitrine may prove to be clinically useful for replacing hypoxic drive during breathing oxygen-rich mixtures, and it will be interesting to determine its mechanism of action on the peripheral chemoreceptors.

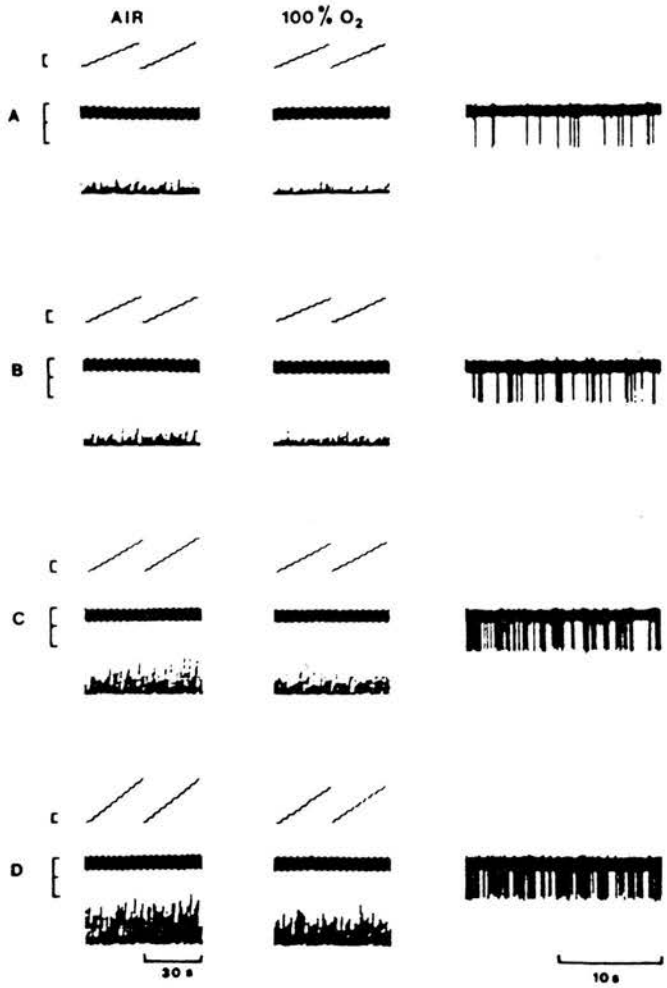
Mechanism of Chemoreception

It seems fair to state at the outset that there is no generally accepted explanation of the mechanisms involved in chemoreception, and there is still considerable debate concerning which of the elements present in peripheral chemoreceptor tissues (cells, nerve fibres, blood vessels, connective tissue) is the primary receptor. Many hypotheses have been advanced to explain how the physiological stimulus to the peripheral arterial chemoreceptors (hypoxia and/or hypercapnia) is transduced into the neural signal carried by sensory fibres to the CNS. The topic is a complex one, partly because of technical difficulties inherent in studying small structures such as the carotid and aortic bodies, and partly because a lot of conflicting evidence has been amassed from experiments, often performed on different species, using a variety of experimental techniques. We propose to examine the different components in the chemoreceptors and then will review some of the main hypotheses, finally finishing this chapter by considering some of the more recent evidence which may suggest new interpretations of existing information, or lines for future research.

Cells of the Arterial Chemoreceptors

Specific chemoreceptor tissue comprises about 50 per cent of the carotid body in cat and rat. The tissues involved are the Type I and Type II cells, blood vessels, nerve fibres and nerve terminals. The Type I cell (see Figure 1.6) is present in the greatest number and is about 10μm in diameter. It has a large dense nucleus, vesicles containing electron-dense granules, an endoplasmic reticulum, Golgi apparatus and microtubules, i.e. all the features associated with secretory cells. The cells are arranged in groups, with a single nerve fibre

Figure 1.5: The Effects of i.v. Infusion of 50 μg (kg^{-1}) min^{-1} Almitrine. Panels show, from above downwards: respiratory half-minute volume 60s after switching to breathing the gas indicated (each breath being represented by a step in the pneumotachograph record); femoral B.P.: counter output in counts s^{-1} for a single carotid chemoreceptor unit. The neurograms to the right of the figure were recorded during oxygen breathing. A is the control, B is 2 and 4 min after starting the infusion, C is 6 and 8 min and D 10 and 12 min after starting the infusion.



Source: McQueen, Price and Ungar, unpublished data.

Figure 1.6: The Ultrastructure of the Carotid Body. T1 – Type I cell; Type II cell; ➤ nerve fibres; ● endoplasmic reticulum; ✱ mitochondria; ★ electron-dense cored vesicles; n.e. nerve endings. Fig 1.6a shows two adjacent Type I cells. Note the mitochondria and electron-dense cored vesicles. Most of the free surface of the Type I cells is covered by attenuated processes of Type II cells (arrows); in some areas however (small arrow heads) the Type I cells communicate directly with the extracellular space. Fig 1.6b and 1.6c illustrate nerve endings. Note how in 1.6b the ending contains mainly mitochondria, whilst those in 1.6c contain many clear cored vesicles. In all three figures (and in Fig 1.2) note the peripheral position of the Type II cells, and the numerous unmyelinated nerve fibres.

Figure 1.6b and c (*contd*)

sending branches to between 10 and 20 Type I cells; a fibre can innervate more than one cell group. The nerve terminals are intimately associated with the cells, there being a synapse or gap of about 30 μm between nerve terminal and Type I cell membrane. Cutting the innervation to cat and rat carotid bodies does not affect the appearance of Type I cells, although the sensory fibres degenerate. In the rabbit, however, section of the sinus nerve causes regression of the carotid body (Kienecke *et al.*, 1981; Tan, Pallot & Purves, 1981 both in Belmonte *et al.*, 1981). Type I cells are also intimately associated with the blood supply, being exposed to the pericapillary space where the cells are only covered by a basement membrane. The Type II cells which partially surround 4–5 Type I cells have not been studied as extensively as Type I cells. They appear to contain carbonic anhydrase and possibly other enzymes. Unlike the Type I cells, Type II cells do not appear to be innervated, but they do encircle numerous small unmyelinated nerve fibres. Pallot (1976 in Paintal, 1976) has demonstrated that some of these unmyelinated fibres in the cat and mouse carotid bodies terminate without forming Type I cell junctions. The major ultrastructural features of the carotid body are illustrated in Figure 1.6.

Various substances have been identified in carotid body cells and the list includes dopamine, noradrenaline, adrenaline, 5-hydroxytryptamine (5-HT, serotonin) and probably acetylcholine in amounts that vary from species to species and even within a species. The fact that these substances are neurotransmitters in other parts of the nervous system has led to their being considered as putative transmitters in arterial chemoreceptors. However, for a long time the carotid body was regarded as a gland, particularly since it is morphologically similar to the chromaffin cell of the adrenal medulla, and there have been suggestions that it may have an endocrine or paraneurone (Fujita & Kobayashi, 1979) function. Pearse (1969) classified the Type I cell in his APUD (amine precursor uptake and decarboxylation) cell series and predicted that they secrete a polypeptide which he named glomin. Recent work using immunocytochemical techniques have established that VIP (vasoactive intestinal polypeptide), substance P, methionine and leucine enkephalin are present in the carotid body and it would not be surprising if more polypeptides are found there. Substance P like material has been identified in cells and nerve fibres of the cat carotid body (Cuello & McQueen, 1980). Whether more than one of the putative neurotransmitters coexist in the cells, and whether the different peptides are stored and released with particular substances requires more detailed

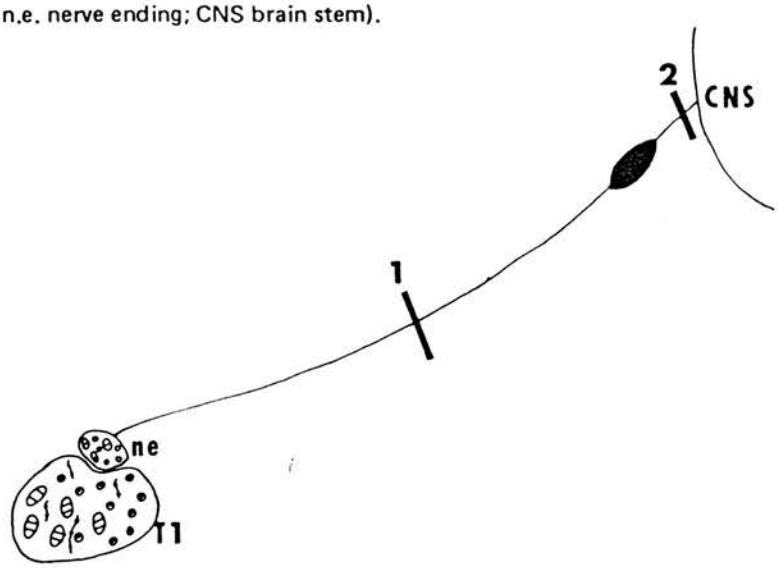
investigation as does the identity of the nerves with which the peptides are associated.

The existence of so many substances in the carotid body, together with evidence based on vesicle size and density measurements, has led to the suggestion that Type I cell sub-types exist. This subject is reviewed by Verna (1979) who casts doubt on the hypothesis that cells may be subdivided on the basis of vesicle size.

Nerve Supply to the Arterial Chemoreceptors

The nerve supply to the arterial chemoreceptors has been most extensively studied in carotid body, but there is no evidence to suggest that innervation of the aortic bodies is appreciably different. There is some evidence that in rats a small proportion of nerve endings associated with Type I cells are sympathetic in origin, (McDonald & Mitchell, 1975) but the overwhelming majority of sympathetic nerve fibres from the superior cervical ganglion innervate the carotid body vasculature. Most of the Type I cell nerve endings are derived from nerve fibres in the carotid sinus nerve, and the classical opinion was that they are the terminations of sensory axons (De Castro, 1928). The evidence for this rested on degeneration studies as illustrated diagrammatically in Figure 1.7.

Figure 1.7: Diagram to Explain the Degeneration Studies. 1 and 2 represent extra- and intra-cranial sections respectively (T1 – Type I cell; n.e. nerve ending; CNS brain stem).



If, after section of the carotid sinus nerve at 1, the nerve endings associated with Type I cells degenerate, then the parent axons run in that nerve. Section of the nerve at 2, central to its sensory ganglion, should differentiate between afferent and efferent nerve endings since the peripheral process of an axon degenerates when severed from its cell body. According to De Castro (1928) section of the carotid sinus nerve at 1 results in degeneration of Type I cell nerve endings whereas section at 2 does not affect the endings.

Physiological studies by Biscoe's group in the late 1960s (reviewed by Biscoe, 1971) have demonstrated efferent or centrifugal activity in the carotid sinus nerve which can be increased by hypoxia, hypercapnia and i.v. adrenaline. In the intact preparation, activation of this efferent pathway decreases afferent activity, and section of the carotid sinus nerve increases chemosensory discharge (see Biscoe, 1971; O'Regan, 1977).

The presence of such a centrifugal pathway in the sinus nerve, and the effects of section of the sinus nerve on chemoreceptor activity, led Biscoe, Lall & Sampson (1970) to repeat the degeneration experiments referred to above (Figure 1.7). They found that three months after section of the IXth nerve between the ganglia and brainstem there was a 60–70 per cent reduction in the number of Type I cell nerve endings; furthermore normal chemoreceptor afferent activity could still be recorded from the sinus nerve. The authors suggested that many of the Type I cell nerve endings represented the terminations of the efferent pathway referred to above. These experiments have been the object of considerable criticism, and subsequent attempts to repeat them have failed (see Verna, 1979). In spite of this it is very difficult to explain the degeneration unless one is to assume that one or other (petrosal or Ehrenritters) ganglion was damaged by Biscoe *et al.* (1970).

This suggestion, which seemed unlikely in view of the normal afferent discharge, may with hindsight prove to be correct. In this respect it is interesting that Willshaw & McAllen (1981 in Belmonte *et al.*, 1981) have provided evidence that the cell bodies of the sinus efferents are located outside the brainstem, in some as yet unidentified site.

Each nerve terminal is characterised by an accumulation of mitochondria and/or clear vesicles (see Figure 1.6). In addition, some endings contain dense-cored vesicles, neurofilaments, neurotubules and glycogen particles. It is presumed that the clear and dense-cored vesicles contain unidentified transmitter substance(s).

The evidence from degeneration studies which purports to establish the nature (sensory or motor) of nerve fibres opposed to the Type I cell

has been conflicting, and part of the difficulty seems to arise from the lack of quantitative data. Morphological studies are unable to establish the function of synapses, but the appearance of the nerve-cell junction sometimes is in accord with a motor (efferent) synapse, with vesicles in the nerve terminal apposed to the cell, sometimes a sensory synapse (vesicles in cell), and occasionally a mixture of the two, referred to as reciprocal synapses (McDonald & Mitchell, 1975; Blakeman & Pallot, 1983 in Pallot, 1983). Experiments performed on mutant mice (Pallot, 1978) have shown quantitatively that in these animals, which have a motor disorder, few endings are found on Type I cells in the carotid body as compared with normal mice, although the mutants have normal chemoreceptor reflexes and this implies that a high proportion of endings are motor, supporting the concept of an efferent pathway. Against this is more recent evidence with radioactive amino acids such as proline which, after injection into the petrosal (sensory) ganglion where the cell bodies are situated, is transported to the carotid body. It is claimed that a substantial proportion of nerve endings on Type I cells in cats are labelled, implying that these are sensory nerve terminals. Such a technique however does not lend itself to quantitative studies.

There is evidence from horseradish peroxidase studies which suggests that only very few efferent fibres course from the CNS in the carotid sinus nerve. Again, part of the problem in interpreting the results arises from difficulties with the methodology and the quantification of results. It will be necessary to establish where the cell bodies of the efferent fibres are located.

Pallot and his colleagues (Morgan, Pallot & Willshaw, 1981; Pallot, Morgan & Willshaw in Belmonte *et al.*, 1981) have recently provided evidence for the existence of an efferent innervation of Type I cells in the cat. They ventilated animals with either 10 per cent or 100 per cent O_2 with one sinus nerve intact and the other cut and then examined the vesicle content of the nerve endings. In all cases after ventilation with 100 per cent O_2 there were more vesicles in the endings from the intact nerve carotid body than the contralateral (sectioned nerve) organ. After 10 per cent O_2 there were fewer vesicles in the nerve endings of the intact nerve carotid body than in the contralateral one. As the stimulus to the carotid bodies themselves is similar, regardless of the state of the nerve, this result indicates that the stimulus acted central to the point of section, and is hence a direct efferent effect on these nerve endings. Parallel purely structural studies (Pallot & Blakeman, 1983 in Pallot, 1983) suggest that on the basis of nerve ending polarity some 50 per cent of endings might be efferents, assuming that the methods

for assessing synaptic polarity in the CNS are applicable to the periphery (see Verna, 1979).

The balance of evidence then, seems to suggest that both afferent and efferent fibres terminate on Type I cells. In view of the serial reconstruction studies (Biscoe & Pallot, 1972; Nishi & Stensaas, 1974) which suggest that most cells receive innervation from a single axon, it may be that afferent and efferent endings are found on different Type I cells. Efferent fibres may also supply blood vessels since it has been shown that sinus nerve stimulation affects carotid body blood flow, an effect which can be prevented by atropine. The question arises as to how physiologically important the efferent pathway to the peripheral chemoreceptors is, as there is certainly no doubt that the sensory pathway can continue operating in the absence of all efferent nerve input to the chemoreceptors. The argument about afferent/efferent fibres in the carotid sinus nerve has led to the compromise suggestion (McDonald, 1980) that carotid sinus nerves can carry impulses in both directions, being antidromically activated by potential changes in the central terminals under certain conditions, but such a mechanism cannot explain the observation referred to above. It is also possible that some of the inhibition of chemosensory discharge seen following stimulation of the sinus nerve results from substances being released from sensory endings by antidromic nerve impulses (e.g. polypeptides).

There are myelinated and unmyelinated fibres in the carotid and aortic nerves and conduction velocity studies show about 66 per cent of carotid chemoreceptor fibres conduct at 5-50 m/s (A fibres – presumed to be myelinated), with the remainder conducting at 0.5-2 m/s (C fibres – unmyelinated). Fidone & Sato (1969) found that the fibres differed in their sensitivity to various stimuli, with A fibres being the most sensitive to acetylcholine (ACh). In contrast, the C fibres of the aortic chemoreceptors have been reported to be much more sensitive to ACh than the A fibres in cats. There may be differences in the two chemoreceptor organs in the responsiveness of their myelinated and unmyelinated fibres to ACh, but Paintal (1971) has criticised the technique used by Fidone & Sato for estimating the conduction velocity of fibres in the very short length of carotid sinus nerve available for experimentation, arguing that their results may be misleading.

Sympathetic Nerve Supply to Arterial Chemoreceptors

Arterial chemoreceptors receive a sympathetic nerve supply, as previously mentioned, and there is general agreement that sympathetic

nerve stimulation can increase carotid chemoreceptor discharge and respiration although this is a somewhat variable effect. Blood flow through the carotid body is reduced by the same procedure and oxygen consumption falls. Cutting the ganglioglomerular (sympathetic) nerves leads to an increase in blood flow through the carotid body. The fact that sympathetic nerves have no effect on chemosensory discharge of the *in vitro* carotid body preparation supports the notion that the sympathetic nerves regulate blood flow in the carotid body.

Apart from effects on chemoreceptor discharge which are secondary to changes in blood flow, there may also be some direct influence on Type I cells. There is morphological evidence that Type I cells in rats receive a pre-ganglionic sympathetic innervation. Sympathectomy has no effect on the electron-dense granules in the Type I cells or on the catecholamine content of cat carotid body (Zapata *et al.*, 1969; Mir, Al Neamy, Pallot & Nahorski, 1983 in Pallot, 1983) but in the rat carotid body sympathectomy causes a 90 per cent reduction in noradrenaline leveis (Mir *et al.*, 1983, in Pallot, 1983).

Blood Vessels

There has already been some discussion concerning blood flow through the carotid body, and it will be necessary to perform further studies *in vivo* in order to compare flow in the aortic and carotid bodies, and to determine how flow is regulated. Meanwhile, it is important to realise that many physiological and pharmacological stimuli are likely to influence blood flow in these organs. Performing experiments *in vitro* may avoid these difficulties, but does introduce other complications, such as the reduced O_2 usage and the inability to sustain chemoreceptor discharge in response to a prolonged stimulus. It has been suggested that responses alter if the carotid body is perfused by saline rather than blood, and this may be due to the greater oxygen-carrying capacity of blood compared with saline, or to the presence of some essential factor in the blood.

Mechanisms of Chemoreception

Various hypotheses have been advanced to explain the mechanism involved in chemoreception, and these will be considered in relation to recent evidence. Biscoe (1971) proposed that free nerve endings in the pericellular space are the chemosensors and the endings on Type I cells are efferent, being involved in modulating sensory activity. The

suggestion is that these fine nerve endings have a high metabolic rate, which is needed to maintain their polarisation, and a large surface to volume ratio. They respond rapidly to hypoxia, a fall in PaO_2 leading to depolarisation of the fibres as a consequence of reduced ion pump (e.g. sodium pump) activity. Sympathetic nerve stimulation reduces oxygen usage by the carotid body, but increases chemoreceptor discharge, and this could be interpreted as meaning that the oxygen supply is locally rate limiting to metabolism, since sympathetically-induced vasoconstriction would reduce flow, leading to chemoreceptor stimulation and reduced oxygen consumption. However, the oxygen consumption of the individual elements of the carotid body is not known, and changes in the oxygen usage of the whole organ may not reflect consumption of the nerve terminals. Further work is needed to characterise these 'free endings'.

Evidence showing that many of the fibres terminating on Type I cells are sensory, has already been referred to, and is obviously not compatible with the free ending hypothesis. Freezing the carotid body causes a permanent loss of Type I and Type II cells, and although the carotid sinus nerve regenerates and the vasculature appears normal, no respiratory reflexes are elicited from the carotid body and nor can chemoreceptor activity be recorded from the carotid sinus nerve. Electrical stimulation of the regenerated nerve does evoke reflex respiratory changes, so demonstrating that the afferent pathway is normal (Verna, Roumy & Leitner, 1981, in Belmonte *et al.*, 1981). The difficulty with such experiments lies in assessing the damage to the nerve endings. Crushing a carotid sinus nerve causes the sensory fibres to degenerate and chemosensory activity is lost. The fibres regenerate, but no chemosensory activity is recorded until the fibres have grown fairly close to the Type I cells (Zapata, Stensaas & Eyzaguirre, 1976), a situation which is similar to that seen in the skin where, following a crush of the sensory nerve innervating the Merkel cells in cats, the regenerating sensory endings show unspecialised sensitivity while the axon is growing, and typical response characteristics are not restored until the Merkel cell-nerve complex is reformed.

It appears from this evidence that chemoreception can only occur when the regenerating fibres come close to the Type I cell – they do not have to be as closely apposed as normal for function to be restored (Zapata *et al.*, 1976) – and this can be interpreted as meaning either that the cells release a chemical (trophic factor) which activates the sensory ending, or the cell causes the ending to become chemosensitive, or vice versa. Kienecker, Knoche & Binmann (1981, in Belmonte *et al.*,

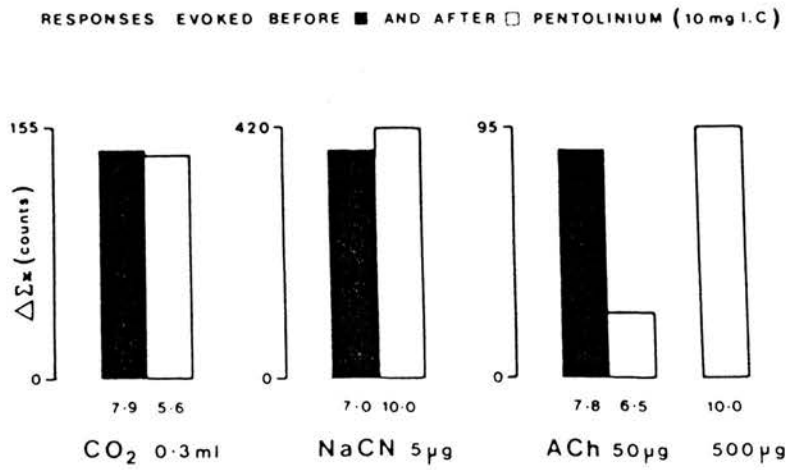
1981) have reported that in rabbits chemosensitivity appears long before the Type I cells are innervated, and it has been claimed that carotid sinus nerve fibres allowed to regenerate into the adventitia of the external carotid artery are chemosensitive (Bingmann *et al.* in Belmonte *et al.*, 1981; Tan, Pallot & Purves, 1981 in Belmonte *et al.*, 1981), but slight chemosensitivity may be a non-specific property of neuromas (see Smith & Mills, 1981 in Belmonte *et al.*, 1981). From this it will be appreciated that the evidence is equivocal and different species have been used by different workers in the studies. Tan *et al.* (1981) seem to provide the best evidence for specific chemosensitivity in neuromas as they demonstrate a recording from a neuroma which is indistinguishable from that of a normal chemoreceptor afferent. Even in this work there is the possibility of reinnervation of miniglomera by sprouts from the regenerating sinus nerve. A useful experiment would be to study the neuroma *in vitro*, when subsequent histology could exclude this possibility.

A further hypothesis based on the sensory endings being the chemosensors is that of Mitchell & McDonald (in Purves, 1975) which proposes that sensory fibres apposed to Type I cells are directly affected by hypoxia or hypercapnia and afferent activity leads to release of a transmitter from the sensory nerve terminal, and the transmitter activates the Type I cell (which is considered to be a dopaminergic interneurone) and dopamine then inhibits the sensory terminal. Morphological evidence for such reciprocal synapses has already been discussed and, again, much hinges on the species and whether or not the nerve endings are chemosensitive. However, the fact that cutting the carotid sinus nerve does not change the levels of known putative transmitters in the carotid body, something which might be expected to occur if sensory fibres contain such a substance argues against this idea.

The hypothesis that physiological stimuli cause the Type I cell to release a chemical (transmitter) which activates the sensory nerve fibres (i.e. the Type I cell is the chemosensor) has been the subject of a great many studies. The problem lies in determining whether chemical transmission occurs and, if it does, establishing the identity of the hypothetical transmitter(s). The oldest candidate, one might even call it classical, is acetylcholine (ACh), and the evidence for and against its role in chemoreception has been reviewed recently (Eyzaguirre & Fidone, 1980; Eyzaguirre, in Belmonte *et al.*, 1981). Briefly, ACh is released in response to physiological stimuli and there appears to be a choline uptake mechanism in Type I cells. The random nature of spontaneous chemosensory discharge could, by analogy with miniature end-plate

potentials of the neuromuscular junction, be due to release of quanta of ACh. However, the properties of fine nerve terminals in the chemoreceptors (cf. Biscoe, 1971) could equally account for the randomness of the normal discharge.

Figure 1.8: Response of Carotid Chemoreceptors (three units counted) to Intra-carotid Injection of 100% CO_2-equilibrated Locke Solution, NaCN and ACh Before and After Administering the Nicotinic Blocking Drug, Pentolinium. The increase in discharge above baseline values ($\Delta\epsilon x$) for each of the responses is submaximal and the baseline discharge in ct/s is given at the foot of each column. After pentolinium the dose of ACh had to be increased by a factor of 10 in order to approximate the pre-pentolinium response, whereas responses to CO_2 and NaCN were not appreciably affected by the nicotinic antagonist.



Source: McQueen, unpublished record from 3.5 kg cat.

ACh levels in the cat carotid body are not affected by cutting either the carotid sinus or the sympathetic nerves, implying that ACh is not stored in nerves. ACh excites all chemoreceptor fibres (e.g. see Figures 1.4, 1.8, Fidone & Sato, 1969), although Paintal (1971) considers it does not affect aortic myelinated chemoreceptor afferents, whereas physiological stimuli do. The major argument against ACh being a chemosensory transmitter in the chemoreceptors has been failure of antagonists to cause any significant reduction in responses to physiological stimuli, although they virtually abolish the excitatory response

evoked by exogenous ACh, and whereas anticholinesterases, such as physostigmine, potentiate exogenous ACh, they have little or no effect on more physiological stimulants (Figure 1.8, and McQueen, 1977, 1980).

Although the pharmacological evidence does not favour a chemoexcitatory role for ACh, it can be argued that, if the concentration of ACh released locally within the carotid body was high compared with that which reaches the site following exogenous administration, or if exogenous ACh acts at an 'extra-synaptic' site, concentrations of drugs used to modify the action of ACh released by physiological stimuli at the intrinsic site may not be adequate. This is difficult to refute entirely, but the fact that very high doses of drugs which penetrate tissues fairly readily, and which are allowed to act for prolonged periods, affect responses to exogenous ACh without having much effect on responses to physiological stimuli, taken together with the observation that large-molecular-weight substances (e.g. horseradish peroxidase) can readily penetrate the chemoreceptor tissues, makes the argument appear improbable.

Results from autoradiographic experiments with labelled α-bungarotoxin (Dinger *et al.*, 1981) show binding sites located on Type I, and possible Type II cells, with no evidence of binding to nerves. The authors equate the binding site for α-bungarotoxin with the acetylcholine nicotinic receptor site, but does bungarotoxin in fact label that nicotinic receptor which when activated causes an increase in chemosensory discharge? The reason for caution is that α-bungarotoxin in the doses used by McQueen (1977) was not very effective at antagonising the excitatory action of ACh on the cat carotid body. It would be interesting to see whether labelled hexamethonium and mecamylamine, established antagonists of the ACh response, bind to the same sites as α-bungarotoxin. If it emerges that there are really no nicotinic receptors on chemosensory nerve endings, or only on those from unmyelinated fibres, it will be necessary to revise considerably the 'cholinergic' hypothesis that ACh released from cells by physiological stimuli depolarises the sensory fibres. The presence of ACh receptors on Type I or Type II cells may mean that ACh released from one cell can influence another cell in the group. In this way, ACh might release one or more chemoexcitatory substances via actions on nicotinic and possibly muscarinic receptors on cells, and these actions would be susceptible to pharmacological antagonists. Physiological stimuli may be acting through several mechanisms to cause chemoreception, such

that loss (or potentiation) of the cholinergic component does not greatly affect the overall response.

ACh may have more than one action on the chemoreceptor complex for there is some evidence that it has an inhibitory role, and indeed chemoinhibition is the predominant effect of ACh on the rabbit carotid body (see Docherty and McQueen, 1979). Some of the inhibitory effects of efferent nerve stimulation of the chemosensory discharge can be prevented by atropine, and this suggests that ACh may be involved in this pathway. Cholinergic receptors may also be involved in control of blood flow through the carotid body.

Intracellular electrophysiological studies have shown that Type I cells are variably affected by physiological and chemical stimuli and fairly insensitive to changes in ionic composition of their environment, although they do respond to changes in temperature and osmotic pressure. Hypoxia, ACh and NaCN have no consistent effect on the membrane potential, sometimes hyperpolarising, sometimes depolarising the cells, yet they invariably increase sensory discharge (see Eyzaguirre & Fidone, 1980). One might suspect that these small cells are rather badly damaged by the electrode because the average resting membrane potential of -20mV seems low and as techniques improve, values nearer to -60mV are being obtained. The variability of cells in their responses to various stimuli may be a consequence of the technical difficulties associated with recording from small cells, or from the fact that they are *in vitro*, but it may also mean that individual cells in a glomerulus differ in their sensitivity to stimuli, perhaps according to the type of receptor(s) they carry. If graded (receptor) potentials are a requirement for the primary receptor element, then the Type I cell does not appear to fit the bill. However, the extent to which the cell membrane potential and resistance are important for the primary transducer element remains to be established. It is also noteworthy that the cells of the arterial chemoreceptors in cats and rats do not seem to be affected by chronic denervation of the sensory nerve, appearing morphologically similar to 'normal' cells and showing similar changes in membrane potential in response to stimulants, although voltage noise is reduced. This contrasts with gustatory chemoreceptors of the tongue, and touch receptors in the skin, where the taste bud cells and Merkel cells disappear following sensory denervation, although in the latter case this is species dependent. In this respect it is interesting that the rabbit carotid body does appear to involute following section of the sinus nerve (Pallot, unpublished; Kienecker, personal communication).

The other putative neurotransmitters in the carotid body (noradrenaline, adrenaline, dopamine and 5-HT) have all been investigated but as yet their role, if any, in chemoreception remains, like that of ACh, to be established. We shall briefly consider evidence relating to dopamine, which is present in the carotid body and released during physiological stimulation of the chemoreceptors. Although there may be species differences, in general low doses of dopamine inhibit spontaneous chemoreceptor activity, whereas higher doses, or lower doses after administration of drugs which block the inhibitory effect, cause chemoexcitation (see Docherty & McQueen, 1978). Dopamine receptors in the carotid body may, therefore, be of two types, inhibitory (DAi) and excitatory (DAe). Respiration in man is reduced by dopamine (Bainbridge & Heistad, 1980). It was suggested by Osborne & Butler (1975) that in the carotid body dopamine is continually released from Type I cells during normoxia and suppresses the tendency of sensory endings to depolarise spontaneously. In hypoxia, dopamine release is attenuated and the chemosensory activity increases. But dopamine release is found to increase during hypoxia, and there is also pharmacological evidence which is not in accord with the hypothesis (see Docherty & McQueen, 1978).

The fact that administration of exogenous dopamine causes a predominately inhibitory effect on carotid chemosensors has led to various proposals concerning the involvement of dopamine in negative feedback and the consensus is that it has a 'modulatory' role, modifying ongoing discharge without being directly responsible for that discharge. In contrast to the inhibitory action, it was suggested over 20 years ago that catecholamines might stimulate sensory nerves in the carotid body (see Biscoe, 1971), and Docherty (1980) recently presented pharmacological evidence which is compatible with endogenous dopamine having an excitatory influence on cat carotid chemoreceptors. Recently, interest in the possible importance of noradrenaline to chemoreceptors has revived and the debate continues as to whether more noradrenaline than dopamine is present in the carotid body; it seems to be species-dependent. One complication of studying the actions of catecholamines *in vivo* is the well-known vascular effects of these substances, and this has led to experiments being performed *in vitro*, where vascular effects are eliminated. However, the extent to which results from the *in vitro* blood-free preparations are directly comparable to those obtained *in vivo* is a moot point as has already been mentioned.

Various other hypotheses exist concerning how the peripheral chemoreceptors work. These include the following: intracellular

bicarbonate ions regulate sensory discharge (see Torrance in Belmonte *et al.*, 1981); Type II cells are the chemosensors, with changes in a low affinity cytochrome enzyme leading to the establishment of a K^+ gradient which can depolarise nerve terminals situated near the Type II cells (Mills & Jöbsis, 1970); Type II cells are chemosensors, which respond to changes in oxygen tension, by mechanically affecting the sensory nerve ending and causing a depolarisation (see Paintal, 1977); changes in cyclic nucleotides, particularly ATP, within chemoreceptor tissue are involved in regulation of chemoreceptor discharge (Anichkov & Belen'kii, 1963) – ATP and adenosine do affect chemosensory activity (see Figure 1.4). These various hypotheses are supported, to a greater or lesser degree by experimental evidence, but to date no single hypothesis has emerged as being generally acceptable, and the involvement of chemical transmission in chemoreception remains to be established.

The variety of hypotheses may mean that different mechanisms within the receptor complex are capable of increasing chemosensory discharge, with the final stage of all the processes being the same. Thus, hypoxia and hypercapnia might act via different cells and/or mechanisms, and there is some evidence to suggest this may be the case, but the final pathway which leads to increased chemosensory discharge could be common, perhaps involving adenylate cyclase or Ca^{2+}. Further studies using ion-sensitive electrodes, tissue slices, cultured cells, and microanalytical techniques should enable a better understanding of the biophysical and biochemical processes to emerge in the near future. It is often assumed that aortic and carotid chemoreceptors are quantitatively similar, but we have already seen some evidence which implies that this may not be the case. Results obtained with one set of receptors, or in one species, should be applied with great caution to the other set, or to other species, lest the basic mechanisms of chemoreception differ.

We have not so far considered the role polypeptides might play in the peripheral chemoreceptors. They do influence carotid chemoreceptor discharge when injected (see McQueen in Belmonte *et al.*, 1981), but because the role of polypeptides in the body has yet to be established, one hesitates to suggest their function in the peripheral chemoreceptors. They may be neurotransmitters or neuromodulators, but could equally well have a neuroendocrine, trophic or other function on cells and/or nerve endings. It will probably emerge that some of the peptides are stored in chemoreceptor cells together with the 'classical' putative transmitters, and may be released along with these substances. Drugs which can specifically affect polypeptides (e.g. antagonists,

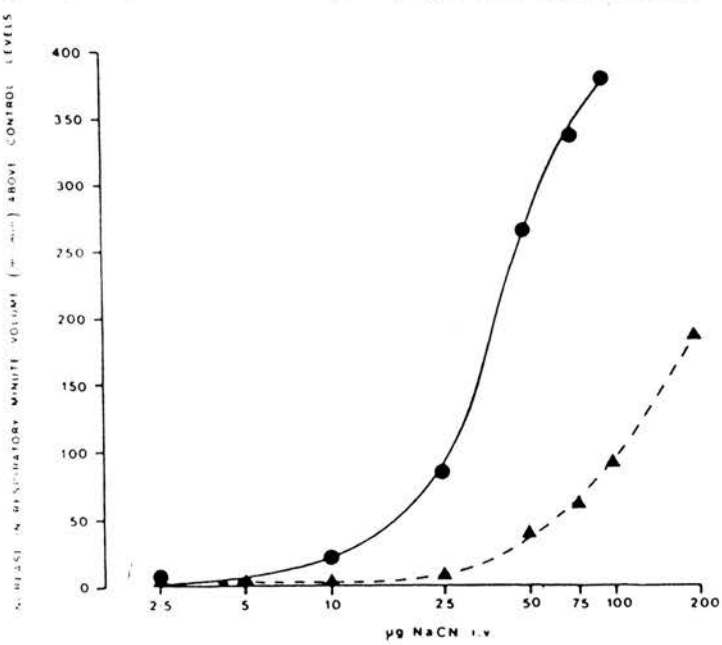
enzyme inhibitors) are needed in order to facilitate study of the physiological role of polypeptides. In the present absence of such drugs, some information may be obtained using specific antibodies to interfere with the peptides. The presence of these various substances in the chemoreceptor tissue may mean that the chemoreceptors are not homogeneous, but rather made up of cell clusters in which the branches of a single nerve fibre are influenced by cells with different characteristics, perhaps releasing different substances in response to physiological and pharmacological stimuli, these substances having excitatory or inhibitory actions, either directly or indirectly mediated (by influencing sensitivity to physiological stimuli), on chemosensory activity. The substances released may, besides influencing the sensory nerves, also affect cells and blood vessels in the vicinity. The integrated input to the CNS from such a glomerulus would be the algebraic summation of the various components and might be subject to efferent feedback.

The role of Type I and Type II cells in chemoreception is problematic – whether the cells release one or more chemical transmitters to affect the sensory nerve endings, or whether cellular activity influences the sensitivity of the nerve endings some other way, remains to be established, as indeed does the more general question concerning which of the chemoreceptor elements is the chemosensor. An alternative proposal is that some of the cells are not directly involved in chemoreception, but have a separate function, perhaps endocrine. In this context it should be remembered that the carotid body was, for a long time, considered to be a gland, and it could be that it has a dual function – sensory organ and gland.

Although two types of chemosensory fibre exist, namely myelinated and unmyelinated, it is not known whether they subserve different functions and act by different mechanisms. Preliminary studies in anaesthetised rats have demonstrated a significant reduction in basal respiration and a loss of chemoreceptor responsiveness in anaesthetised animals which were treated with capsaicin neonatally (Figure 1.9). This drug causes a permanent loss of the majority of unmyelinated sensory afferent nerve fibres and the results observed could be the consequence of a destruction of the unmyelinated chemoreceptor afferents. The reduced respiratory responsiveness may be due to a reduced peripheral chemoreceptor input to the CNS, or some effect in the CNS consequent to destruction of the primary afferent terminals. It has been suggested that substance P is a neurotransmitter of chemoreceptor afferents in the nucleus tractus solitarius of the rat (Gillis *et al.*, 1980), and capsaicin does cause a destruction of SP-containing fibres. However, it is also able

to affect unmyelinated sensory fibres containing other polypeptides, so the results cannot be interpreted exclusively in terms of a loss of SP-containing fibres. The study of animals treated neonatally with capsaicin may allow an assessment of the relative contribution made to respiratory reflexes by myelinated and unmyelinated chemoreceptor afferents, and may also be useful for investigating the physiological role of polypeptides in the peripheral chemoreceptors.

Figure 1.9: Averaged Increase in Respiratory Minute Volume in Pentobarbitone Anaesthetised Rats During the 20s Period Following i.v. Injection of NaCN (log_{10} scale) in Vehicle-treated (–, n=3) and Capsaicin-treated (---, n=3) Animals. Lines were fitted to the data by eye. The rats were seven months old when used for the study and had been injected with either capsaicin (50 mg kg^{-1} s.c.) or drug vehicle 50:50 polyethylene glycol/saline) two days after birth. The body weights of the two groups were not significantly different, but capsaicin-treated animals had a significantly lower ($P < 0.01$) respiratory minute volume than the controls (93±8.1 compared with 154±6.7 ml min^{-1}) and their respiratory frequency was also reduced (51±1.2 against 59±2.1 breaths min^{-1}).



Source: McQueen and Cervero, unpublished observations.

In conclusion, the influence of the peripheral arterial chemoreceptors on respiration has been investigated and is fairly well understood, whereas the identity of the chemosensor(s) and the mechanism(s) of transduction within the peripheral chemoreceptors remain to be established. The pathophysiology of the aortic and carotid bodies should be studied since respiratory and other disorders might follow from changes in peripheral chemoreceptor function, perhaps as a result of 'sclerosis of the organs' (Gomez, 1908) or as a consequence of repeated stimulation.

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CHAPTER 8

Pharmacological aspects of putative transmitters in the carotid body

DANIEL S. McQUEEN

Department of Pharmacology, University of Edinburgh Medical School, Edinburgh EH8 9JZ, Scotland

Contents

1. Introduction

Pharmacological studies involving the use of drugs such as selective agonists, antagonists, enzyme inhibitors, biosynthesis inhibitors and neurotoxins proved very valuable in helping to establish that chemical transmission occurs in the *motor* nervous system, so it is not too surprising that, despite the structural differences between motor and sensory synapses, a similar approach was adopted as part of the attempt to determine whether transmitter (generator) substances are involved in the *sensory* process of chemoreception, i.e. the mechanism whereby the physiological stimulus is transduced into a neural signal by the peripheral arterial chemoreceptors. Most investigators have chosen to study the carotid body chemoreceptors, and consequently a considerable amount of information concerning the pharmacology of these sensory receptors has accumulated – there is even a book on the topic (Anichkov and Belen'kii, 1963). Although pharmacological studies have made an important contribution to our knowledge of the carotid body, they have not provided clear evidence for the involvement in chemoreception of any particular chemical, in contrast to the situation with motor nerves. This is not to deny the existence of evidence favouring chemical transmission in arterial chemoreception (e.g. see Eyzaguirre and Zapata, 1968a), but the consensus appears to be that the case for chemical transmission has yet to be proved, and it should be noted that hypotheses exist which purport to explain chemoreception without invoking chemical transmission (e.g. Paintal, 1967).

The aim of this chapter is to review the pharmacology of putative transmitters in the carotid body and to provide some insight into the problems encountered in studying the pharmacology of this organ and interpreting the results obtained. The question of what is meant by the term "putative transmitter" immediately arises and, in order to avoid entering into a somewhat semantic discussion about neurotransmitters, co-transmitters, modulators and neurohormones, a fairly catholic interpretation will be applied in the present context. The involvement of acetylcholine (ACh) and the catecholamines in chemoreception will be considered in detail, but some attention will be given to all the substances shown in Fig. 1 since they are present within the carotid body and reputed to function as transmitters in other parts of the nervous system. We shall also consider the actions in the carotid body of some substances, listed in Table 1, which may be transmitters in other parts of the nervous system, even though their presence in, or absence from, the carotid body remains to be established. It is not always possible to detect and identify endogenous substances when present in low concentrations within a very small structure such as the carotid body. For example, pharmacological studies on the question of whether or not acetylcholine is a chemosensory transmitter began over 40 years ago, yet it is only recently that convincing evidence for the presence of this putative transmitter in the carotid body has been obtained using gas chromatography–mass spectrometry (GC–MS) (Fidone et al., 1976), a technique that had not been devised when the first suggestion that ACh might be a transmitter involved in chemoreception was made (Schweitzer and Wright, 1938).

Peptides have been included in the list of putative transmitters somewhat

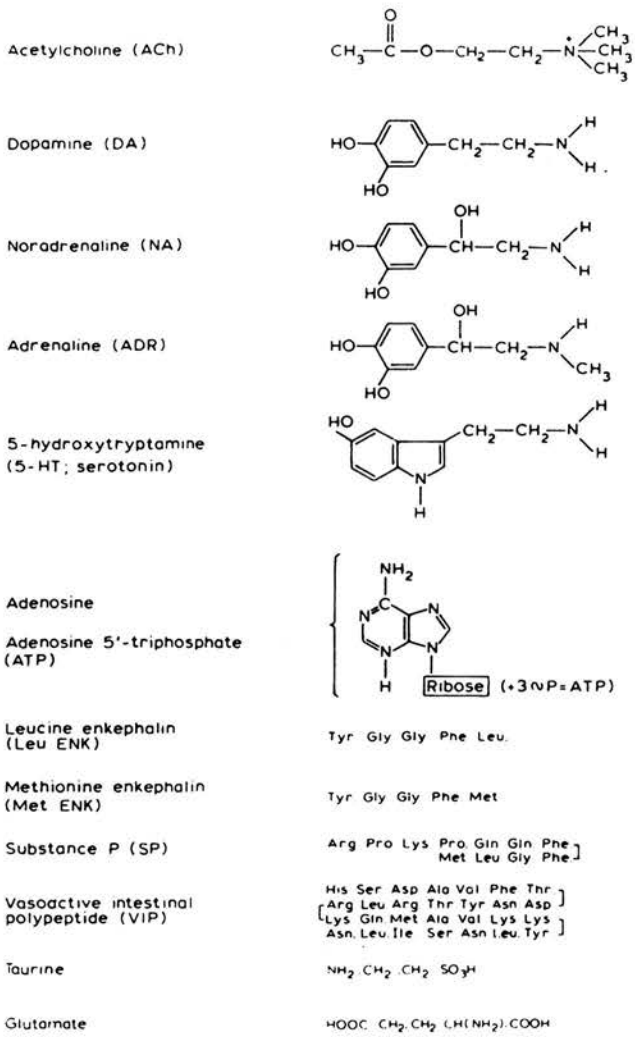


Fig. 1. Chemical structures of putative transmitters known to be present in the carotid body.

TABLE I

SUBSTANCES WHICH EVIDENCE SUGGESTS MAY BE TRANSMITTERS IN OTHER PARTS OF THE NERVOUS SYSTEM, BUT WHOSE PRESENCE IN THE CAROTID BODY IS NOT ESTABLISHED

Amino acids: γ-aminobutyric acid (GABA)
Prostaglandins (PGs)
Histamine
Various polypeptides, including
cholecystokinin octapeptide (CCK-8), neurotensin, glucagon, carnosine, somatostatin, bradykinin, bombesin, angiotensin II, β-endorphin (β-END), vasopressin (ADH)

tentatively as there is only limited evidence in favour of their having a transmitter function, and it is not unreasonable to state that their precise role is unknown at present. However, some of them do appear to be located in the carotid body and are capable of modifying chemoreceptor activity (McQueen, 1981), so it seemed worth considering their pharmacology in more detail than that of the others.

1.1. Theoretical considerations

Before making a start on the pharmacology of individual substances, consideration will be given to general problems encountered in studying the pharmacology of carotid body chemoreceptors. The carotid body is a receptor complex which comprises nerve endings, type I cells, type II cells, blood vessels and connective tissue. It is not known which of the receptor elements (nerve endings or cells) is the primary site for chemoreception (see Eyzaguirre and Fidone, 1980). In addition to afferent or sensory nerve fibres which course in the carotid sinus nerve and terminate near some of the cells, the organ is innervated by efferent or motor fibres which originate from the superior cervical ganglion (sympathetic nerves) and the carotid sinus nerve. Efferent nerves terminate near blood vessels and some cells, and are capable of modifying chemosensory activity (see Chapter 11). Chemical transmission in efferent nerve pathways is well established, so it is very probable that some of the putative carotid body transmitters are associated with efferent nerves. Potential sites of drug action within the carotid body are shown schematically in Fig. 2, and it will be appreciated that many problems are involved in trying to determine where within this complex structure an exogenously administered putative transmitter, or any other drug, is acting to affect chemosensory activity, particularly since effects at more than one of the sites (sensory nerves, motor nerves, cells, vasculature) could be involved. The possibility also exists that functionally dissimilar sites within the carotid body may employ the same transmitter substance. Further complications arise if more than one substance is involved in transmission at a single site (co-transmission), because studies on the putative transmitter role of one substance in the absence of the other may be misleading.

The cells of the carotid body have been likened to those of the adrenal medulla on the basis of their morphology, and adrenal medullary cells are known to release substances (catecholamines and opioids) which have a hormonal function. The carotid body used to be thought of as a gland, and was even called the carotid gland, but the convincing demonstration of its chemosensory properties (see Heymans and Neil, 1958) has led to a more or less complete abandonment of the endocrine concept. However, Pearse (1969) classified the type I cell in the APUD series of endocrine cells and it could be that, in addition to its undoubted chemosensory function, the carotid body does have an endocrine function. This has important implications for the pharmacological study of putative transmitters in the carotid body since, by analogy with the adrenal medulla, substances such as noradrenaline, adrenaline and possibly opioids could have an endocrine (or paracrine) rather than a transmitter function, or they may even exert a dual action. Most studies on the carotid body are geared to

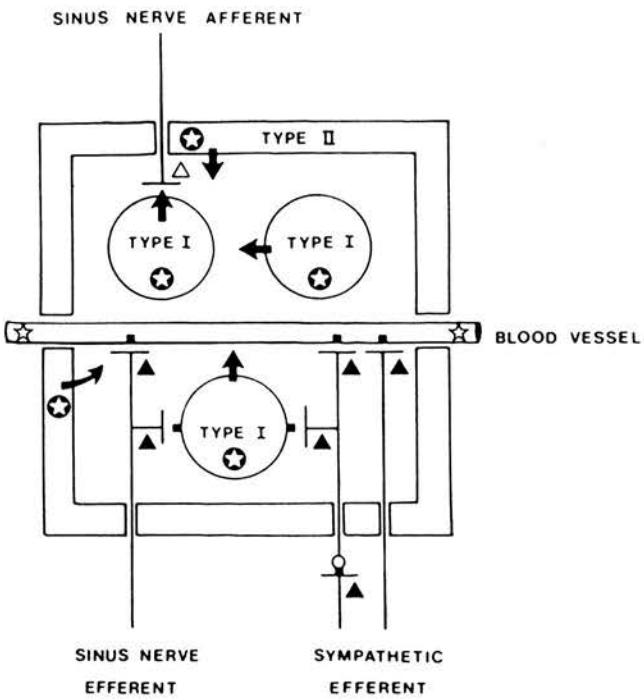


Fig. 2. Schematic representation of the carotid body "receptor complex" showing some potential sites of drug action, namely: △, sensory nerve endings; ▲, motor nerve endings; ■, postsynaptic receptors; ✪, receptors on type I or type II cells that control the release of substances (arrows) which might affect other cells, nerves, or blood vessels; ☆, receptors in blood vessels. Note that not all the cells are innervated, and that there are very few ganglion cells within the carotid body.

measuring changes that occur within seconds of administering a drug, and they are not really suitable for detecting drug-induced effects that take several minutes or hours to develop.

1.2. Practical considerations

Having considered some of the theoretical problems associated with carotid body pharmacology, the intention is now to review the practical aspects of the subject. Pharmacological studies on the carotid body have generated quite a lot of conflicting evidence: Why is this? Part of the answer to the question is that the small size of the carotid body in commonly studied species does not facilitate pharmacological studies, but the real problem lies in the fact that a variety of techniques involving different investigators, animal species, drug doses and methods of evaluating and expressing results have been used, such that it is difficult and sometimes downright impossible to make a meaningful comparison of the evidence presented by

different workers. If the carotid chemoreceptors are heterogeneous, i.e. comprising different populations which do not share the same pharmacological properties, this might also explain why conflicting evidence arises.

Some of the methods that have been used in studying the pharmacology of carotid body chemoreceptors are listed in Table 2, and in general terms they fall into two categories, namely those that utilize electrophysiological techniques, and those that do not. Electrophysiological methods have provided a great deal of information about the pharmacology of carotid chemoreceptors, but it has to be appreciated that there are disadvantages associated with their use. In early studies it was difficult to distinguish between chemoreceptor and baroreceptor potentials in the multiunit recordings made from the mixed function carotid sinus nerve. As techniques improved multiunit chemoreceptor recordings were obtained, but these are difficult to interpret because it is not known if the population being recorded is constant, and quantification of responses evoked by drugs is problematic. Single unit (single functional fibre) studies overcame most of these problems, but meant that only very small samples of the total fibre population (which in a sinus nerve runs into the thousands) could be investigated. The sinus nerve contains chemoreceptor A and C (fast and slow conducting fibres, probably myelinated and unmyelinated respectively) fibres and these may not have identical pharmacological properties (Paintal, 1967; Fidone and Sato, 1969). Single units are most liable to be recorded from the large diameter (A) fibres since it is technically very difficult regularly to record from small (C) fibres (Iggo, 1960). Consequently, single unit studies tend to be biased towards those units which are most easily recorded, i.e. those which are spontaneously active and associated with larger nerve fibres. Performing several experiments will provide a larger sample, but it will still be a very small and biased one. A compromise is to record 3–4 units which can be separately identified from their amplitude, rise time and shape, then counted by using a pulse height discriminator operating in conjunction with a computer. With this technique drugs can be studied on a larger population than is possible using the single unit method, and it is likely that some of the smaller units recorded will be C fibres. Studies which have compared the drug-induced responses of single and multiple units show them to be remarkably homogeneous (e.g. see Goodman, 1974; McQueen, 1977). Nevertheless, the problem of sample bias remains, and it is actually very difficult to establish the conduction velocity of a fibre in the carotid sinus nerve because of the short length of nerve between stimulating and recording electrodes (see Paintal, 1971), so with the methods commonly used it is not known what type of fibre is being studied. A rough idea can be obtained from the duration of the action potential when bipolar recording electrodes are used because slower conduction is generally associated with a longer duration (but see Iggo, 1958). Histology does not help to determine whether the active fibre was myelinated because even a small filament from which a single unit is recorded contains several fibres, some of which are inactive, and it is impossible to establish from which of these the electrical activity emanated. Incidentally, the term "single fibre", as used in relation to extracellular recordings, is rarely meant literally.

In vitro carotid body preparations have much to offer the pharmacologist who

TABLE 2

METHODS USED IN STUDYING THE PHARMACOLOGY OF THE CAROTID BODY

Method	*Comments*
Study of reflex respiratory, vascular, or other changes following drug administration in the whole animal (see Heymans and Neil, 1958; Anichkov and Belen'kii, 1963)	Study of motor changes provides only limited information about the action of drugs on carotid body receptors. Complications arise from other actions of the drug, the anaesthetic agent, and secondary changes (e.g. in blood gas tensions, lung inflation reflex, blood pressure) which can modify the primary chemoreceptor reflex. Integration in the CNS makes it difficult to relate output (reflex change) to input (drug action on chemoreceptors). However, the method is fairly easy to perform and does provide some information concerning the actions of drugs and physiological stimuli on the whole intact receptor population, although small changes in chemosensory discharge are unlikely to be detected.
Electrophysiological recording of chemosensory discharge in the whole animal and close-arterial drug administration to the carotid body (see Heymans and Neil, 1958; McQueen, 1977)	Recording action potentials in the carotid sinus nerve avoids some of the problems associated with reflex studies and can provide quantitative data concerning drug actions on the receptor complex. Activity in efferent nerves can be eliminated, and secondary influences can be minimised by careful monitoring and control of blood gas tensions, temperature, blood pressure and pH; anaesthetic agents do not exert much direct influence on sensory endings. The disadvantage is that this method does not reveal where within the receptor complex a drug is acting, and the sample of fibres studied is a limited and biased one (see text); recording activity from cell bodies in the petrosal ganglion may enable studies to be performed on carotid chemoreceptors with identified conduction velocities. Vascular effects of drugs may complicate interpretation of results; however, many putative transmitters have vascular effects, and this has not prevented them from being studied in the central and peripheral nervous systems of intact animals.
Electrophysiological recording of chemosensory discharge in vitro (see Eyzaguirre and Zapata, 1968a)	Removing the carotid body from the animal and superfusing it with an appropriate physiological solution provides a constant environment, avoids vascular complications, and enables the concentration of drug reaching the receptors to be predicted – unlike the situation in vivo. The problem of bias in the sample of units recorded remains, and it is not possible to tell where within the complex the drug is acting. Drugs take longer to act in vitro as compared to in vivo, and their effects last longer; those pharmacokinetic differences between preparations may give rise to dissimilar results, and it is perhaps relevant to note that fast or dynamic responses are one of the characteristic features of the carotid chemoreceptors in vivo. There is evidence to suggest that the carotid body does not function as well in vitro as it does in vivo (see O'Regan, 1981), and this may be partly due to the absence of a blood-borne factor (Joels and Neil, 1968).

TABLE 2 (*continued*)

Method	*Comments*
Electrophysiological recording in vitro; Intracellular studies (see Eyzaguirre et al., 1975; Eyzaguirre and Fidone, 1980)	Extracellular studies on the generator potential, and intracellular studies on identified chemoreceptor cells take one much nearer to the receptor. The use of tissue slices, such as elegantly employed by Eyzaguirre, offers the prospect of direct application of drugs to cells and nerve endings, as has been done at the neuromuscular junction (Kuffler and Yoshikami, 1975). Also noise analysis of potentials recorded in single cells and fibres (e.g. Hayashida and Eyzaguirre, 1979; Hayashida et al., 1981) may reveal how putative transmitters affect ion channels, as may studies with ion-sensitive electrodes (e.g. Acker, 1978) and the patch clamp technique. Problems associated with using in vitro preparations, discussed above, and those associated with damage to cells by the relatively large microelectrodes remain to be resolved. Sample bias arises because it is more likely that larger rather than smaller cells and fibres will be recorded. The effects of drugs on cell bodies of chemoreceptor fibres can be studied using preparations of the nodose or the petrosal ganglia.
Pharmacological studies on chemoreceptor neurones and cells in tissue culture (see Pietruschka et al., 1977; Acker and Pietruschka, 1977)	This method would appear to offer great scope for neuropharmacological studies, such as those described above, but also for receptor-labelling experiments and biochemical investigations. It remains a moot point, however, whether studies on isolated individual elements of the carotid body complex provide information which is relevant to those elements in the intact animal; but in principle it seems desirable to study the functioning of the basic units, then to start investigating how these are modified in the more complex structure. One might predict that pharmacological studies on subcellular elements will be performed in the future.
Various (see Christie, 1933; Cuello and McQueen, 1980; Dinger et al., 1981a)	Various methods have been used to locate and identify putative transmitter substances in the carotid body. These include bioassay, biochemistry, immunohistochemistry, histofluorescence, electron microscopy and GC–MS. Receptor-binding sites can also be identified using autoradiography and other techniques. These methods can be used to study drugs which either mimic or modify the actions of putative transmitters on the chemoreceptors. It may be possible to use 2-deoxyglucose to determine which cells in the receptor complex are metabolically active and to investigate whether physiological stimuli and drugs modify their activity.

wishes to characterize receptors for putative transmitters either by determining affinity constants for selective agonists and antagonists or using receptor-binding techniques. Intracellular recording techniques may enable the actions of drugs on ion channels to be studied. However, in common with all other biological systems, the question of whether the isolated preparation retains essentially the same properties it had in vivo is paramount; this gets harder to answer the more the original structure is broken down in vitro into its constituent parts and the integrity of the system is destroyed.

Most of the methods employed have made a contribution to our understanding of carotid body pharmacology, and it is likely that pharmacological studies on the carotid body will continue to use a wide variety of techniques. The problem is to find ways of correlating the results obtained in different preparations. A standard pharmacological tool used for evaluating drug effects is the dose–response curve, and although it means having to quantify responses and study drugs at different dose levels, its use does provide objective evidence concerning the effects of drugs on the carotid body. Qualitative evidence, in contrast, is subjective and can be misleading; single-dose studies can be difficult to evaluate, particularly if the drug is used at a dose which produces a response that is either near threshold or is supramaximal. In chemoreceptor studies it may be necessary to employ single doses, but in that event it should be established that the dose used is one which produces a response within the central region of the dose–response curve.

Chemosensory discharge is irregular, and consequently it is often difficult to distinguish between weak drug-induced effects and spontaneous changes in discharge. Injecting the drug vehicle, generally Locke solution or normal saline, also causes a transient decrease in discharge due to the low P_{CO_2} of the solution (Landgren et al., 1952); this can be largely avoided by bubbling the wash solution with 5% CO_2 in air (see Fig. 12) and injecting small volumes. Chemoreceptors are also sensitive to temperature (e.g. McQueen and Eyzaguirre, 1974), so solutions to be injected close-arterial to the carotid body should be pre-warmed to blood temperature. Recent reports suggest that carotid body chemoreceptors are sensitive to changes in osmotic pressure, both in vitro and in vivo (Gallego et al., 1979; Gallego and Belmonte, 1979). Since some of the drug solutions *injected* close-arterial to the carotid body are hyperosmotic (see Table 3), the question of the extent to which the osmolality of the solution modifies the response naturally arises. Hyperosmotic mannitol solutions injected in 3 times the volume normally administered i.c. by McQueen (1977) have no appreciable effect on spontaneous chemoreceptor discharge (Fig. 3) nor do they affect responses evoked by physiological or pharmacological stimulants. Hyperosmotic sucrose tends to cause a slight delayed increase in discharge lasting for about 30 sec. These findings accord with older reports that injections of hyperosmotic sodium chloride solutions have no marked effect on chemoreceptor activity (e.g. Landgren et al., 1952). Gallego and Belmonte (1979) considered that the decrease in chemoreceptor discharge they observed following *infusion* of hyperosmotic sucrose in vivo, which contrasted with the increase found to occur in vitro by Gallego et al. (1979), was probably due to vascular effect of the solutions.

TABLE 3

OSMOTIC PRESSURES OF SOME CONCENTRATED DRUG SOLUTIONS COMMONLY INJECTED IN A VOLUME OF 0.1–0.3 ml CLOSE-ARTERIAL TO THE CAROTID BODY (McQueen, unpublished data)

Drugs were dissolved in either Locke solution (L) or 0.9% aqueous sodium chloride solution (saline, S) and osmotic pressure was measured using an osmometer (Advanced Instruments).

Drug	*Concentration*	*Osmotic pressure (mOsm)*
Acetylcholine iodide	500 μg/ml (L)	297
Sodium cyanide	50 μg/ml (L)	295
100% CO_2-equilibrated Locke solution		334
Nicotine hydrogen tartrate	50 μg/ml (L)	306
5-HT creatinine sulphate	100 μg/ml (L)	295
Dopamine hydrochloride	50 μg/ml (L)	293
Adrenaline hydrochloride	100 μg/ml (S)	291
Adenosine	100 μg/ml (L)	296
ATP, disodium	100 μg/ml (L)	299
Mecamylamine hydrochloride	10 mg/ml (L)	386
Atropine sulphate	10 mg/ml (L)	335
α-Flupenthixol dihydrochloride	10 mg/ml (S)	315
Substance P	100 μg/ml (L)	306
Met-enkephalin acetate	100 μg/ml (S)	288
Histimine acid phosphate	1 mg/ml (S)	306
GABA	1 mg/ml (S)	309
Taurine	1 mg/ml (S)	308
Phenylbignanide	1 mg/ml (L)	330
Locke solution (drug vehicle)		295
Saline (drug vehicle)		298

2. *Putative transmitters*

2.1. Acetylcholine

2.1.1. Historical review

Many pharmacological studies have been undertaken to test the "cholinergic hypothesis" that ACh is released within the carotid body by physiological stimuli and acts to excite chemosensory nerve endings. Only a brief review will be made in this chapter since full details can be obtained from the reviews by Anichkov and Belen'kii (1963), Eyzaguirre and Zapata (1968a), Biscoe (1971), and Eyzaguirre and Fidone (1980).

Early studies suggested that the pharmacology of the carotid body is very similar to that of ganglion cells in autonomic ganglia, particularly with regard to the excitatory action of ACh, and this reinforced the notion that cells and nerve endings of the carotid body are functionally akin to synapses, possibly using ACh as a transmitter. Eyzaguirre's Loewi-type experiments in vitro (Eyzaguirre et al., 1965) demonstrated that an hypoxic carotid body releases a chemical which is capable of

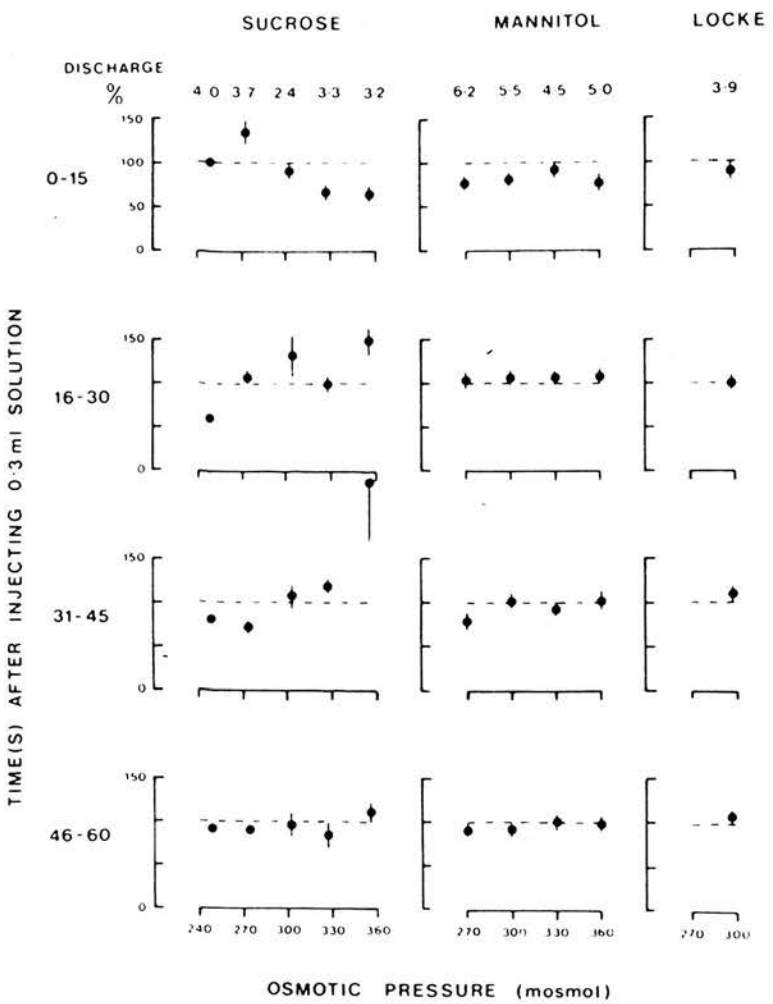


Fig. 3. Data from 3 cats showing the effects on spontaneous discharge of injecting (i.c.) mannitol or sucrose solutions of different osmolality. Animals were anaesthetized with pentobarbitone, artificially ventilated and paralyzed with gallamine (see McQueen, 1977, for full details). Discharge in individual experiments was averaged over 15 sec periods and expressed as a percentage of the 15 sec period immediately preceding the injection (i.e. control = 100%; mean absolute values given above the figure in ct/sec). Pooled data are shown as mean ±S.E.M. (McQueen, unpublished observations.)

evoking increased chemosensory activity in a "down-stream" carotid body preparation. This occurred even when the carotid body which was rendered hypoxic had been chronically denervated, an observation which implies that the chemical originates from cells, not nerves, in the carotid body. Further evidence was obtained which showed that the increase in discharge of the down-stream carotid body could be potentiated by the anticholinesterase drug physostigmine (eserine) and prevented

by the ganglion-blocking drug mecamylamine (Eyzaguirre and Zapata, 1968b). An ACh-like material was detected in the cat carotid body by Eyzaguirre et al. (1965) who used bioassay methods, and the presence of ACh in the carotid body was confirmed by Fidone et al. (1976) using the technique of GC–MS. Chronically denervated carotid bodies contain the same levels of ACh as normal carotid bodies, and chronic superior cervical ganglionectomy does not affect the ACh levels. These findings suggest that ACh is present in cells with the carotid body complex, and a high affinity choline uptake mechanism has been shown to be present in carotid body type I and, possibly, type II cells (Fidone et al., 1977). All this evidence apparently favours the suggestion that ACh is a transmitter or generator substance in the chemosensory mechanism.

However, some of the evidence concerning the putative transmitter function of ACh in the carotid body can be queried. For example, are the techniques used for measuring ACh levels within the carotid body capable of distinguishing between a low concentration of ACh that might be present in nerve-endings and a very much higher concentration associated with chemoreceptor cells? Because it is difficult to show the presence of ACh in tissues, indirect methods have been used. But indirect evidence, such as high affinity choline uptake by cells, is not proof of a cholinergic function in vivo; additional evidence is required. It would be desirable to show the distribution of choline acetyltransferase (CAT) within the carotid body, but at present there is no specific antibody to CAT which could be used reliably in immunocytochemical studies. Interpretation of results obtained using the carotid body in vitro depends on whether the carotid body is functioning normally in this type of preparation, and as discussed in an earlier section this is a moot point.

The strongest evidence against ACh being "the" chemosensory transmitter comes from pharmacological studies with nicotine antagonists such as hexamethonium, mecamylamine and dihydro-β-erythroidine. Briefly, these demonstrate that the blocking drugs can greatly reduce or abolish the excitatory action of exogenous ACh on the chemoreceptors, but have little, if any, effect on responses to physiological stimuli such as hypoxia and hypercania or to pharmacological stimuli such as sodium cyanide (see Figs. 4 and 6) (Douglas, 1952; Eyzaguirre and Zapata, 1968a; McQueen, 1977; Bisgard et al., 1979; Eyzaguirre and Fidone, 1980). Some of the evidence is conflicting, and McQueen (1977) suggested that this may be because different experimental preparations which do not share the same pharmacological and physiological properties have been used. Also much of the evidence is qualitative and, consequently, difficult to evaluate objectively. In addition, certain ganglion-blocking drugs (e.g. hexamethonium) appear to act by blocking ion channels which are opened by the ACh–receptor interaction, whereas others (e.g. mecamylamine) compete with ACh for receptor sites and apparently have little or no direct influence on ion channels (e.g. see Rang, 1981). One can speculate about the implications of such a situation in the carotid body. Suppose the physiological stimuli activate the same ion channel(s) as ACh, but by a non-cholinergic mechanism: some blocking drugs would affect reponses evoked both by ACh and the physiological stimuli, whereas antagonists such as mecamylamine would inhibit ACh-induced responses, but have little effect on responses to physiological stimuli. Such a scheme would help to explain some of the conflicting evidence, and seems worth investigating.

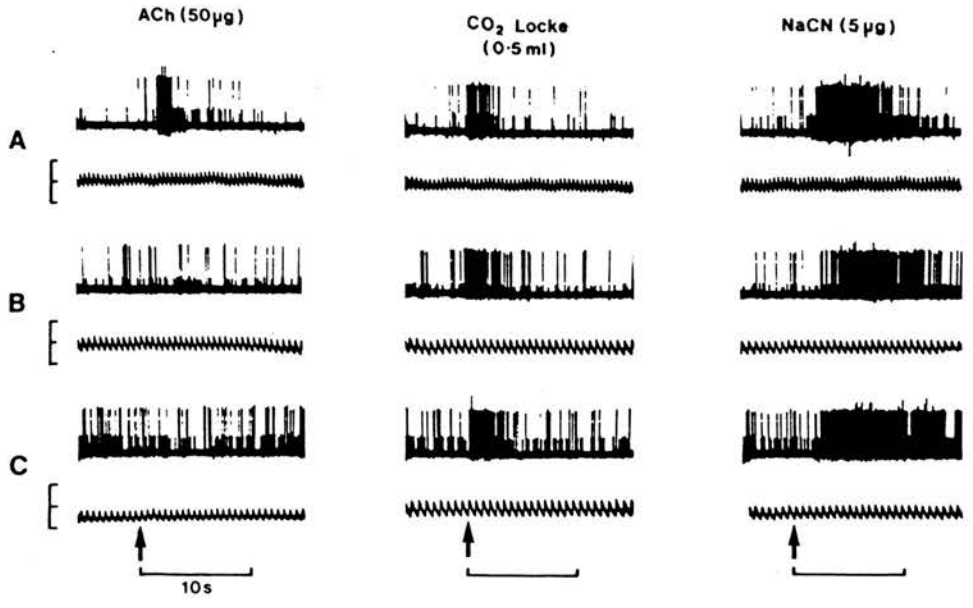


Fig. 4. Recording of carotid chemoreceptor discharge showing responses to intracarotid injections (arrows) of ACh, Locke solution equilibrated with 100% CO_2, and NaCN: A, controls; B, after mecamylamine 1 mg/kg i.c. (dextran solution infused i.v. to maintain blood pressure); C, after the addition of atropine (1 mg/kg i.c.) together with a further dose of mecamylamine. The cat was artificially ventilated, paralyzed with gallamine and the ganglioglomerular nerves cut. BP calibration 0–100–200 mm Hg. (McQueen, unpublished observations.)

The failure of the nicotinic receptor-blocking drugs to reduce appreciably chemosensory responses to physiological stimuli when used in doses capable of very substantially reducing the effect of exogenous ACh could be due to insufficient blocking drug reaching the receptor site. It has been proposed that there are two types of ACh receptors, those in the cell-nerve cleft, and those outside the cleft which are referred to as "extrasynaptic" receptors (Eyzaguirre and Zapata, 1968a). Endogenous ACh, probably in high concentrations, would act on the "intrinsic" receptors and blocking drugs would not penetrate well and so not reach an effective concentration at the intrinsic receptor site. Exogenous ACh acts on extrasynaptic receptors and its action can be antagonized by blocking drugs because the receptor is more accessible. It is difficult to refute completely this suggestion, but some points can be raised which cast doubts on its validity. The barrier preventing blocking drugs from diffusing into the "synaptic" cleft would have to be very substantial given the very high concentrations applied close-arterial to the carotid body and the long time allowed for equilibrium to be reached, yet evidence shows that the enzyme horseradish peroxidase (mol. mass 40 000) rapidly penetrates all regions and intracellular spaces in the rat carotid body (Woods, 1975), and it is a much larger molecule than any of the blocking drugs. Furthermore, it remains to be established whether 'synaptic' and/or extrasynaptic ACh receptors are in fact present on sensory nerve endings (see also Torrance, 1974).

Nerve endings of unmyelinated afferent nerve fibres can be directly excited by ACh (e.g. Douglas and Ritchie, 1962), whereas those of most myelinated fibres are relatively insensitive to the drug. Excitation by ACh need not mean that ACh is involved in the normal functioning of sensory nerve endings, and it has been shown that the effects of exogenous ACh on certain sensory receptors in the skin can be prevented by excess nicotine or by nicotine antagonists without affecting responses to the physiological stimulus (Brown and Gray, 1948; Gray and Diamond, 1957). Thus, actions of ACh on sensory nerve endings may be "non-specific", although if ACh receptors are in fact present on sensory nerves (see below), one might ask what their physiological role is. Paintal (1967) presented evidence from studies on cat *aortic* chemoreceptors which is consistent with the scheme just described, namely that unmyelinated fibres were excited by ACh whereas myelinated fibres were not: both types responded to physiological stimuli. Fidone and Sato (1969), in contrast, reported that ACh can excite both types of chemoreceptor fibre in the cat carotid sinus nerve. As already mentioned, it is difficult to measure conduction velocity in the sinus nerve, and Paintal (1971) has criticized the method used by Fidone and Sato. However, the discrepancy between the results may reflect a real difference between aortic and carotid chemoreceptors in their responsiveness to ACh.

Experiments with radiolabelled α-bungarotoxin in cats show that binding occurs at sites on chemoreceptor cells but not on nerves in the carotid body (Dinger et al., 1981a). The question is whether α-bungarotoxin selectively labels those nicotinic ACh receptors involved in the chemoexcitatory response to ACh, and here caution is needed because α-bungarotoxin does not greatly affect chemosensory responses to ACh in the cat (McQueen, 1977). Even if α-bungarotoxin selectively labels the nicotinic receptors on cells, the apparent absence of receptors on the nerves may be due to a failure of the toxin to bind to nicotinic receptors on nerves or, alternatively, the technique may not be able to resolve the binding to receptors on nerves. Further studies are needed to investigate the matter, particularly since it has obvious implications for the synaptic–extrasynaptic ACh receptor hypothesis referred to above. It is often assumed that ACh acts directly on nerve endings in the carotid body because its effects on chemosensory discharge are so rapid in onset. However, if ACh were to act by releasing a transmitter from chemoreceptor cells, or in some other way indirectly affect chemosensory discharge, it is conceivable that a delay of 20–100 msec is all that might be involved. Such a delay would not be detected by most of the techniques currently used for studying chemoreceptor activity.

In addition to evidence that nicotine antagonists which penetrate tissues well (e.g. mecamylamine, dihydro-β-erythroidine) do not appreciably affect responses to physiological stimuli (e.g. McQueen, 1977, 1980b; but cf. Nishi and Eyzaguirre, 1971), it has also been shown that other drugs such as anticholinesterases and inhibitors of ACh biosynthesis, which might be expected to affect endogenous ACh, have only slight effects on the responses to physiological stimuli (see Douglas, 1954; McQueen, 1977; but cf. Eyzaguirre and Zapata, 1968a). The cumulative evidence does not, on balance, appear to lend much support to the cholinergic hypothesis, which is not to deny that some evidence favouring the hypothesis does exist. To conclude this introductory section it can be said that *proof* of a physiological role for

ACh in the carotid body is lacking. Although it may turn out that ACh is not "the" sensory transmitter substance, there is evidence to suggest that ACh may function in the transduction process, perhaps acting as a co-transmitter or neuromodulator (see Eyzaguirre and Fidone, 1980) and maybe affecting more than one site. Consideration of ACh's pharmacology, free from the influence of the cholinergic hypothesis, may provide a clue as to its function in the carotid body.

2.1.2. Agonist and antagonist drugs which have been used to characterize the ACh receptor

These drugs could be called cholinergic agonists and antagonists, although purists would argue that the term "cholinergic" should be confined to nerves or transmission and not used for drugs. There is no doubt that receptors for ACh exist in the carotid body because injected ACh clearly affects chemoreflex activity and chemosensory discharge, the best-known effect being the chemoexcitation evoked by ACh in most species studied (see Fig. 4) (Heymans and Neil, 1958; Anichkov and Belen'kii, 1963). However, chemoinhibition is the predominant effect in rabbits (see Fig. 5) (Docherty and McQueen, 1978a, 1979). Similar results are obtained from in vivo and in vitro carotid body preparations, including chemoinhibition in vitro with rabbit carotid body (Monti-Bloch and Eyzaguirre, 1980), although responses to ACh in vivo are generally more rapid in onset and shorter-lasting than those in vitro. This temporal differences may be due to the speed at which ACh gains access to the receptor site(s), but the fact that responses are qualitatively similar in vivo and in vitro suggests that vascular actions in vivo are unlikely to be responsible for most of the effects of ACh; they also occur too rapidly. When intracellular potentials are measured, however, it is found that some chemoreceptor cells are hyperpolarized, some depolarized and some

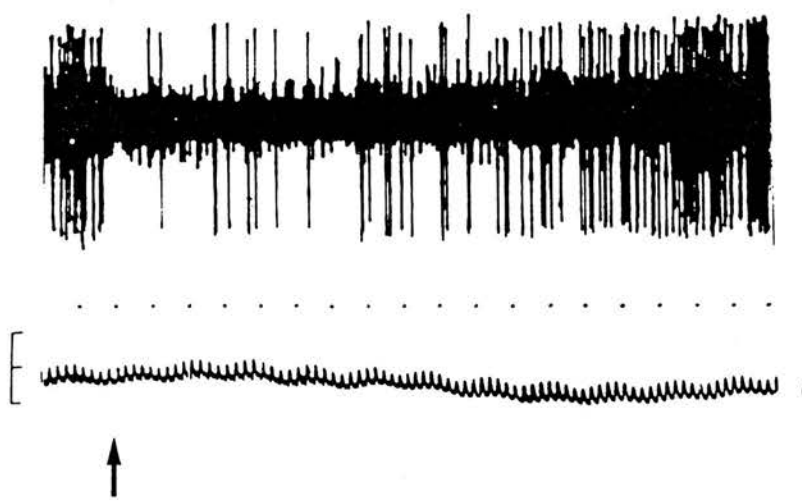


Fig. 5. Chemoreceptor discharge recorded from a rabbit carotid sinus nerve showing the effect of injecting (arrow) ACh 25 μg i.c. BP calibration 0–100–200 mm Hg; time marker 1 sec. (Modified from Docherty and McQueen, 1978a.)

unaffected by ACh (see Eyzaguirre and Fidone, 1980). Are these differences real, and if so, do different types of ACh receptor exist on chemoreceptor cells, producing opposing effects on cell membrane potential when activated? Or is there only one type of receptor, but different cell populations? How much of the change in membrane potential is secondary to, or modified by, actions of substances released by ACh in the carotid body complex, as opposed to direct actions on an ACh receptor?

Considerations of Fig. 2 suggests various sites at which ACh might act, and by analogy with other parts of the nervous system one could predict that effects of ACh will probably result from actions on muscarinic receptors, nicotinic receptors, or a mixture of the two. There is no reason for excluding the presence of a novel type of ACh receptor in the carotid body (see later), but it is simpler to start by investigating the established receptors. ACh acts at both muscarinic and nicotinic receptor sites (i.e. it is not selective), so selective agonists and antagonists have been used to help characterize the ACh receptor(s) present in the carotid body.

Chemoexcitation evoked by ACh appears to result from actions on a nicotinic ACh receptor, as has been demonstrated in a variety of preparations by various workers using agonists such as nicotine, lobeline and suberyldicholine (Heymans, 1955; Anichkov and Belen'kii, 1963; Eyzaguirre and Zapata, 1968a; McQueen, 1974). The effect can be reduced or abolished, depending on the dose of antagonist and that of the agonist, by nicotine antagonists such as hexamethonium, mecamylamine, D-tubocurarine and dihydro-β-erythroidine (e.g. Figs. 4 and 6) (Dontas and Nickerson, 1956; Byck, 1961; Eyzaguirre and Zapata, 1968a; Sampson, 1970; McQueen, 1977, 1980b). It appears that the ACh receptor responsible for chemoexcitation is more akin to that in ganglia than that at the neuromuscular junction (Anichkov and Belen'kii, 1963; McQueen, 1980b), but no affinity constant has yet been determined, and it could turn out to be a different type of receptor. Transient weak chemoexcitation is evoked in rabbits by some nicotinic agonists, a response which can be reduced or abolished by ganglion-blocking drugs (see Docherty and McQueen, 1979; Monti-Bloch and Eyzaguirre, 1980). It may be reasonable to conclude that a nicotinic receptor is involved in the response, but it has to be noted that the nicotinic agonist suberyldicholine, which is a very effective stimulant in cats (McQueen, 1974), has no consistent effect on chemoreceptor discharge in rabbits in vivo (Docherty and McQueen, 1979). This could mean that if a nicotinic ACh receptor is present in the rabbit carotid body, it differs from that in the cat's carotid body. Further studies are needed to investigate the matter.

Chemoinhibition evoked by ACh in rabbits is mimicked by the selective muscarinic agonist bethanechol (Docherty and McQueen, 1979) and pilocarpine (Monti-Bloch and Eyzaguirre, 1980) and substantially reduced by atropine, so it appears to involve a muscarinic ACh receptor. However, in cats pilocarpine does not depress spontaneous chemosensory discharge, as is illustrated in Fig. 6 which shows responses evoked by a spectrum of cholinergic agonists ranging from nicotine to muscarine. It can be seen that although atropine alters the time course of some responses, the overall effect is unaltered, whereas addition of mecamylamine virtually abolishes the excitation evoked by several of these agonists, indicating that the effect resulted from activation of nicotinic receptors (see also McQueen, 1978). ACh does not cause any appreciable

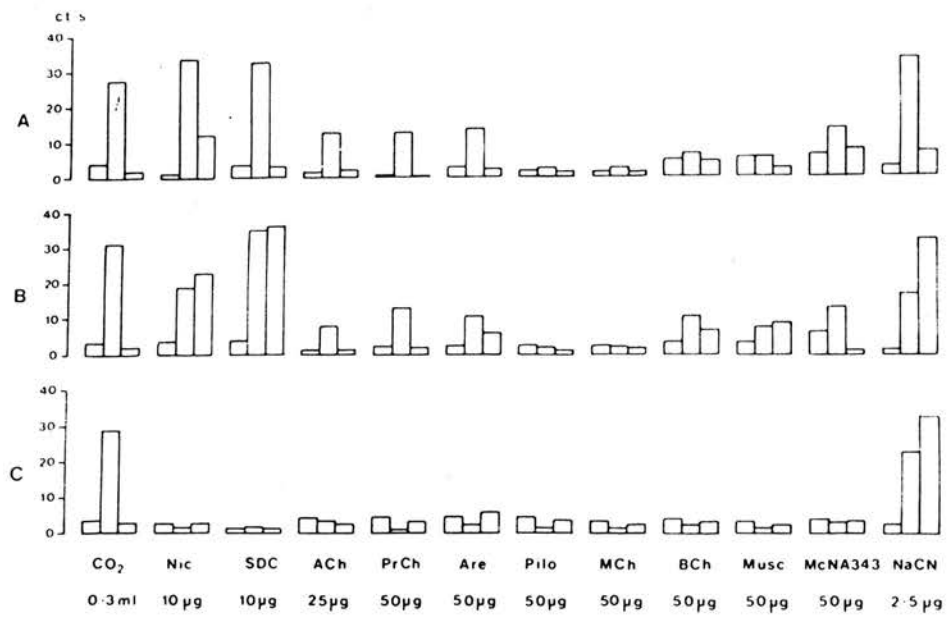


Fig. 6. The actions of submaximal doses of various substances on chemoreceptor discharge (2 units) recorded during a single experiment on a pentobarbitone anaesthetized artificially ventilated paralyzed cat in which the ganglioglomerular nerves had been cut. For each substance 3 blocks are shown. The first represents the background discharge, the second the discharge averaged over the 5 sec period immediately following the i.c. injection, and the third is the discharge averaged over the next 5 sec, all in ct/sec. Drugs included nicotine (Nic), suberyldicholine (SDC), propionylcholine (PrCh), arecoline (Are), pilocarpine (Pilo), methacholine (MCh), bethanechol (BCh), muscarine (Musc). A, controls; B, after atropine 1 mg/kg i.c.; C, after mecamylamine 1 mg/kg i.c. + 5 mg/kg i.v. (McQueen, unpublished observations.)

depression of "spontaneous" chemoreceptor discharge when injected into a non-atropinized cat shortly after a dose of mecamylamine sufficient to abolish the chemo-excitatory response (Fig. 4). This experiment incidentally demonstrates that changes in chemosensory discharge are not secondary to direct effects of drugs such as ACh on the vasculature since the fall in systemic blood pressure, and presumably actions of ACh on carotid body blood vessels, are unaffected by mecamylamine. It has been noted that chemoreceptor responses to sodium cyanide in cats can be potentiated by nicotine-blocking drugs (e.g. Dontas and Nickerson, 1956; Byck, 1961; McQueen, 1977, 1980b) and while this may be the result of some non-specific action of the blocker, it could be interpreted as meaning that endogenous ACh can act via nicotinic receptors to depress discharge. Might there be two populations of nicotinic receptors in the carotid body, one associated with chemoexcitation, the other with chemoinhibition?

Stimulating the carotid sinus nerve antidromically causes inhibition of spontaneous chemoreceptor discharge recorded from a strand of the nerve, and also increases blood flow through the carotid body (Neil and O'Regan, 1971). According to Goodman (1973) this chemoinhibition is secondary to vascular effects and can be

abolished by atropine, but Neil and O'Regan (1971) found that whereas atropine blocked the vascular effects, the inhibition of chemosensory discharge remained and can, in fact, be obtained even in the absence of blood flow (see O'Regan, 1977). Belmonte and Eyzaguirre (1974) favoured a vascular explanation for the chemoinhibition because it was not observed when stimulating the carotid nerve of the in vitro cat carotid body preparation, but they also reported that atropine was not very effective at blocking the chemoinhibition in vivo. It has been proposed by Sampson et al. (1975, 1976) that ACh or some substance whose actions are blocked by atropine is released from sinus nerve efferent fibres and indirectly inhibits chemosensory discharge by releasing catecholamines from type I cells. It is not the intention to enter into a debate on the efferent pathway (see Chapter 11 for that), but rather to point out that circumstantial evidence exists which can be interpreted as meaning that ACh is involved in chemoinhibition (see above).

Further studies are needed to characterize the ACh receptor(s) present within the carotid body, and intracellular recording techniques could be used to investigate quantitatively the actions of selective cholinergic agonists and antagonists on chemoreceptor cells and nerve endings. The possibility that ACh might be acting on a receptor which is neither muscarinic nor nicotinic needs to be considered, particularly as Kehoe (1972) has demonstrated in Aplysia that ACh receptors on certain neurones are stimulated by arecoline but unaffected by muscarine, nicotine, atropine, hexamethonium or curare. However, ACh does not appear to have any effect on spontaneous carotid chemosensory discharge in cats treated with mecamylamine and atropine (e.g. see Figs. 4 and 6), so its actions can be explained in terms of nicotinic and muscarinic ACh receptors, at least according to the available electrophysiological evidence. Furthermore, although arecoline has an excitatory action on cat chemoreceptors, this can be blocked by mecamylamine, so presumably results from actions on nicotinic receptors (Fig. 6).

2.1.3. Other drugs: anticholinesterases, biosynthesis inhibitors and neurotoxins

Acetylcholinesterase and pseudocholinesterase (butyrylcholinesterase) are both present in low concentrations in the cat carotid body, pseudocholinesterase being predominant (Hollinshead and Sawyer, 1945; Koelle, 1950; Biscoe and Silver, 1966). There is general agreement that anticholinesterase drugs potentiate the effects of ACh on chemosensory discharge, but have no influence on nicotine-like drugs such as carbachol which are not destroyed by cholinesterase (see Anichkov and Belen'kii, 1963; McQueen, 1977). Results obtained with physostigmine (eserine), a drug which inhibits both types of cholinesterase, during studies on the effects of ACh and methacholine, led McQueen (1978) to suggest that acetylcholinesterase is located close to the site(s) at which nicotinic agonist drugs act to increase chemosensory discharge. Ballard and Jones (1971) used electron microscope techniques and found that acetylcholinesterase is localized to peripheral axons in cat carotid body, but conflicting evidence and problems with the methodology mean that it is difficult to be certain about the precise localization of acetylcholinesterase in the carotid body (Jones, 1975).

If ACh is a transmitter whose action is rapidly terminated by cholinesterase in the carotid body, it might be expected that natural stimuli capable of evoking intense

chemoreceptor discharge should, in the presence of an anticholinesterase drug such as physostigmine, lead to paralysis of the system. This is what happens in sympathetic ganglia (Feldberg and Vartiainen, 1935). Such an effect does not seem to occur in cats (McQueen, 1977), although it should be noted that Eyzaguirre and Zapata (1968a) reported that the response of chemoreceptors to stimulation by acid is reduced by an excess of ACh, particularly in the presence of choline. As previously stated, there is disagreement about whether or not anticholinesterases potentiate submaximal responses to natural stimuli. Undoubtedly much depends on the technique used, but in general terms it appears that potentiation is a variable and inconsistent phenomenon (see Anichkov and Belen'kii, 1963) and on balance there is more evidence against it occurring than there is for it.

The high affinity choline uptake mechanism that exists in the carotid body appears to be associated with chemoreceptor cells because (a) chronic denervation of the carotid body has no measurable effect on choline uptake and (b) radiolabelled choline is concentrated mainly in chemoreceptor cells (Fidone et al., 1977). Low concentrations of hemicholinium-3, a drug which inhibits choline uptake, reduced or abolished synthesis of [^{3}H]ACh from [^{3}H]choline by the carotid body in vitro. Treatment with hemicholinium-3 followed by prolonged periods of hypoxia (to exhaust stores of ACh) reduces responses evoked by the chemoreceptor stimulant sodium cyanide but has little effect on responses to ACh, and this occurred both in vivo and in vitro (Eyzaguirre and Zapata, 1968c; Nishi and Eyzaguirre, 1971; Eyzaguirre and Nishi, 1974). Results from cat experiments in vivo also showed that hemicholinium-3 reduces average chemoreceptor discharge evoked by sodium cyanide, but revealed that the integrated or overall response was increased (McQueen, 1977). The latter finding could mean that endogenous ACh depresses the chemoreceptor response to sodium cyanide, or else is involved in regulating the temporal pattern of the response. On balance, however, the weight of evidence suggests that hemicholinium-3 reduces responses to stimulants such as sodium cyanide, which is compatible with ACh having a chemoexcitatory function. Having said that, it is necessary to stress that none of the evidence is really satisfactory and further studies are needed to correlate alterations in chemoreceptor responsiveness with changes in ACh output following hemicholinium-3 treatment in vivo. Even then it will be necessary to determine whether ACh has more than one function in the carotid body (e.g. inhibition via an efferent pathway; excitation via an afferent pathway) since hemicholinium-3 might affect these differentially.

Results from studies using various substances which can modify the storage or release of ACh have provided evidence concerning the cholinergic nature of certain neural pathways. For example, high concentrations of magnesium ions depress the release of ACh from cholinergic nerves whereas lanthanum ions provoke its release. As far as the carotid chemoreceptors are concerned, high magnesium reduces the membrane potential of chemoreceptor cells (Eyzaguirre and Fidone, 1980) and increases chemosensory discharge (Eyzaguirre and Zapata, 1968b) while lanthanum ions cause an increase in spontaneous chemosensory discharge (Roumy and Leitner, 1977). These findings are consistent with ACh being involved in chemoexcitation, but the posssibility exists that they could be due to actions of the ions on some other

transmitter in the carotid body since changes in chemoreceptor activity have not yet been correlated with ACh output (see above).

There do not appear to have been any detailed studies on the effects of venoms and toxins on the carotid chemoreceptors, which is perhaps not too surprising given the nature of the 'drugs'. Botulinum toxin depresses the release of ACh from cholinergic nerves, and is fairly specific, but black widow spider venom, which releases ACh, may release other transmitters besides ACh. The snake venom β-bungarotoxin has been used to cause release of ACh at cholinergic junctions; release of ACh is followed by block of transmission and a reduction in choline uptake. β-Bungarotoxin had no appreciable influence on responses of the cat carotid body chemoreceptors in vivo to sodium cyanide (McQueen, 1977), which could be taken as evidence against ACh being involved in chemoexcitation evoked by that stimulant. These experiments can be criticized because it was not known whether the venom reached an effective concentration in the carotid body, or for how long it was present. It will be necessary to measure ACh output from the carotid body in order to reach meaningful conclusions regarding the actions of β-bungarotoxin, or any of the venoms and toxins already mentioned, on chemosensory activity.

2.1.4. Conclusions

Much of the pharmacological evidence is inadequate or conflicting and does not enable one to conclude whether or not ACh functions as a transmitter that is involved in carotid body chemoreception. This state of affairs is rather disappointing, particularly since very similar opinions were expressed nearly 30 years ago (Daly, 1954; Douglas, 1954), but it simply reflects the facts of the matter. ACh appears to be present in the carotid body and presumably has some function there, so why has it not been possible to determine its role? Part of the answer is that fundamental problems remain to be solved. For example, it has yet to be established whether the chemoexcitation evoked by ACh results from direct or indirect actions on chemoreceptor cells, nerve endings, or a combination of the elements in the carotid body complex. This situation is not peculiar to ACh – the primary transduction site in the carotid body has also yet to be established for physiological stimuli (Eyzaguirre and Fidone, 1980). A particular deficiency is the lack of data relating output of (identified) ACh to chemosensory discharge or changes in membrane potential of chemoreceptor cells under the influence of different physiological and pharmacological stimuli.

Further studies are obviously needed. It is probable that no single method will establish what ACh does in the carotid body and a variety of techniques will be used in future pharmacological studies. It is vital, therefore, that investigations should provide quantitative evidence concerning drug effects on chemoreceptors in order to minimize the possibility of conflicting evidence arising from qualitative evidence, something which has dogged this field in the past. Similarly, it has to be appreciated that results obtained in different species, or in the same species under widely differing experimental conditions, may not be directly comparable.

As for the types of experiment that might be performed in the future, one can envisage studies on carotid body slice preparations in which potentials are recorded from identified chemoreceptor cells and from closely apposed nerve endings while

drugs are applied locally by ionophoresis (e.g. applying techniques used by Kuffler and Yoshikami (1975) on snake and frog neuromuscular junctions; see also Eyzaguirre et al. (1975) on the carotid body). Such studies may help to establish which is the primary receptor element and provide information concerning the site(s) of action of ACh. Carotid body preparations in vitro offer many advantages to the pharmacologist. For example, vascular complications are eliminated, known concentrations of agonists and antagonists can be used, substances released during chemoreceptor stimulation can be collected from the perfusion fluid, and studies with microelectrodes are greatly facilitated. It will, however, be necessary to show the extent to which functioning of chemoreceptors in vitro differs from that in vivo, something which might be difficult to do, particularly if the cells are heterogeneous.

Much more work can be done to characterize the ACh receptor(s) present in the carotid body, to determine which element(s) of the chemosensory complex these receptors are associated with, and to establish what biophysical and/or biochemical changes result from receptor activation. Existing techniques, or refinements of these, could be used for some of the studies, but new techniques will have to be adopted or devised for others. Modern analytical methods such as GC–MS could be used to investigate the possibility sometimes encountered in the literature (e.g. Heymans, 1955) that it is a choline ester with similar properties to ACh which is involved in chemoexcitation – rather than, or in addition to, ACh. Following this line of thought, if ACh in the carotid body functions together with a co-transmitter (a fashionable concept, e.g. ACh-VIP in sweat glands – Hökfelt et al., 1980a), then it is possible that drugs which affect one of the putative transmitters without influencing the other might provide results which do not necessarily reveal the physiological role of the "transmitter". The phenomenon of co-existence of transmitters (Hökfelt et al., 1980b) could be explored in the carotid body using modern histochemical and immunocytochemical methods, although the fundamental problem of identifying ACh in tissues still has to be solved. It is worth noting that various polypeptides, including VIP-like material, are present in the carotid body, something which will be discussed in a later section.

It should be borne in mind that ACh may have actions in the carotid body that are independent of any role(s) it may have in chemoreception. Finally, the points discussed in this section have related to ACh, but most of them apply in general terms to any putative transmitter.

2.2. Catecholamines

2.2.1. Historical review

This field has recently been reviewed (see Mills et al., 1978; Eyzaguirre and Fidone, 1980; Fidone et al., 1980) and these works should be consulted for a more detailed discussion than is possible here. The carotid body contains the catecholamines dopamine, noradrenaline and adrenaline, the absolute amount of each amine varying from species to species, and even within a species. For example, Zapata et al. (1969) found dopamine levels which were double those of noradrenaline in the cat carotid body, whereas in the same species Mills et al. (1978) found nearly 5 times more

noradrenaline than dopamine. Much appears to depend on the methodology used for estimating catecholamines and the state of the tissues when removed from the animal but while the question of whether it is dopamine or noradrenaline which predominates awaits a definitive answer, there is general agreement that levels of adrenaline in the carotid body are usually much lower than those of the other two amines.

What is known about the function of catecholamines in the carotid body? One possibility is that they are transmitters associated with postganglionic sympathetic nerves. However, the finding that chronic superior cervical ganglionectomy has no appreciable effect on the catecholamine content of cat carotid bodies (Zapata et al., 1969) argues against such a pathway accounting for the greater part of the catecholamines. In rats about 50% of noradrenaline is lost after chronic sympathectomy, but dopamine levels are largely unaltered (Hanbauer and Hellström, 1978). Even in this species a substantial part of the noradrenaline present in the carotid body is not associated with sympathetic nerves. Chronic sectioning of the carotid sinus nerve has no appreciable effect on the catecholamine content of the carotid body (see Chapter 1) which implies that if catecholamines are associated with fibres in this nerve the levels must be very much lower than those in the carotid body. Most of the catecholamines are located in dense-cored vesicles in type I cells, although whether different amines are stored in the same vesicles, in separate vesicles in the same cell, or in separate vesicles in separate cells is unclear. Some evidence based on size and electron density of vesicles has been interpreted as meaning that at least two varieties of type I cell exist (e.g. Lever et al., 1959; McDonald and Mitchell, 1975), but the quality of fixation, state of maturity of cells and lack of quantification of vesicle size makes it difficult to assess the situation (see Chapter 1).

It has also been proposed that catecholamines in the type I cells are released by efferent (motor) activity in the carotid sinus nerve (see Biscoe, 1971; Eyzaguirre and Fidone, 1980; also Chapter 11) and act as modulators of chemosensory discharge. Noradrenaline was found to cause rather variable effects on chemoreceptor discharge, and the increase it generally evoked was considered to be secondary to reduced blood flow through the carotid body caused by vasoconstriction (Joels and Neil, 1963). No chemoexcitation was observed in vitro (Eyzaguirre and Zapata, 1968a), which supports this interpretation (but cf. Biscoe, 1965). In contrast, dopamine was found to depress spontaneous and evoked chemosensory discharge when injected close-arterial to the carotid body in many species (Black et al., 1972; Zapata, 1975; Docherty and McQueen, 1978b, 1979) by a non-vascular action (Sampson et al., 1976) and it therefore became a likely candidate for the role of inhibitory transmitter or modulator (e.g. Sampson, 1972; Mitchell and McDonald, 1975). Dopamine is released from some carotid body preparations during hypoxia (see Fidone et al., 1980) and dopamine levels in rat carotid bodies fall during hypoxia, even when the carotid sinus nerve is cut (Hanbauer and Hellström, 1978).

Osborne and Butler (1975) hypothesized that in the normal resting state dopamine is tonically released from type I cells and suppresses the spontaneous depolarization of afferent nerve endings; in hypoxia dopamine release is attenuated and consequently afferent nerve activity increases. This hypothesis seemed not to accord with the finding that dopamine output increases during hypoxia (even in denervated

carotid body) nor with results reported by Eyzaguirre and Zapata (1968a) showing that, 24–48 h after injecting one or more doses of reserpine sufficient to reduce catecholamine levels in the cat carotid body substantially, the carotid chemoreceptors continue to function normally both in vivo and in vitro. Cowan and Greene (1965) found that the respiratory response to hypoxia, but not that to sodium cyanide, was almost completely abolished in a reserpinized cat, and a slow i.v. infusion of noradrenaline restored the response to hypoxia. This experiment is difficult to evaluate because central actions of reserpine may affect the reflex pathway, but it is interesting that a difference between reflex responsiveness to hypoxia and cyanide was obtained. Experiments with reserpine are difficult to interpret because (a) levels of 5-HT, and possibly other substances, as well as catecholamines could be affected and (b) a reserpine-resistant pool of amines may remain allowing normal functioning even when tissue levels of the amines have been greatly reduced.

Pharmacological studies have been undertaken in an attempt to characterize the catecholamine receptors which are present in the carotid body and to obtain some insight into the role of catecholamines in this organ. Results from some of the experiments will now be considered.

2.2.2. Dopamine

The predominant action of low doses of dopamine on cat and rabbit carotid bodies in situ is a depression of both spontaneous and evoked chemosensory discharge (Fig. 7) (Sampson, 1972; Nishi, 1975; Zapata and Llados, 1977; Docherty and McQueen, 1978b, 1979; Lahiri et al., 1980). It was reported that excitation is obtained in response to injected dopamine in dogs (Jacobs and Comroe, 1968; Black et al., 1972), but Bisgard et al. (1979) found this only occurs with high doses – depression of discharge is seen following low doses. Chemoexcitation can sometimes also be observed in cats and rabbits as a delayed effect (cf. the rapid response in dogs – Bisgard et al., 1979) following the rather variable inhibition induced by high doses of dopamine, or with lower doses after a dopamine antagonist has been administered. When cat or rabbit carotid bodies are studied in vitro chemoexcitation is more commonly obtained (Zapata, 1975; Eyzaguirre and Monti-Bloch, 1980), and it appears that in these preparations the dopamine receptor mediating inhibition becomes desensitized (Zapata, 1975), something which does not occur in vivo (Llados and Zapata, 1978a). However, dopamine hyperpolarized chemoreceptor nerve endings of cat and rabbit carotid bodies in vitro, which would be associated with chemoinhibition, and tachyphylaxis to this effect was not observed in the experiments of Sampson and Vidruk (1977). The results obtained in vitro, therefore, need to be interpreted carefully. So too do those from the carotid body in situ because, as several workers have pointed out, alterations in chemosensory discharge may be secondary to vascular changes caused by the drug. However, responses obtained during the first 10–20 sec after a close-arterial injection are unlikely to be due to vascular changes because such changes take time to occur and the carotid body requires more time to react to them. In addition, many drugs with pronounced actions on the vasculature have very little effect on chemosensory discharge in cats unless blood pressure falls below about 60 mm Hg. The real stumbling block, whatever the preparation, lies in

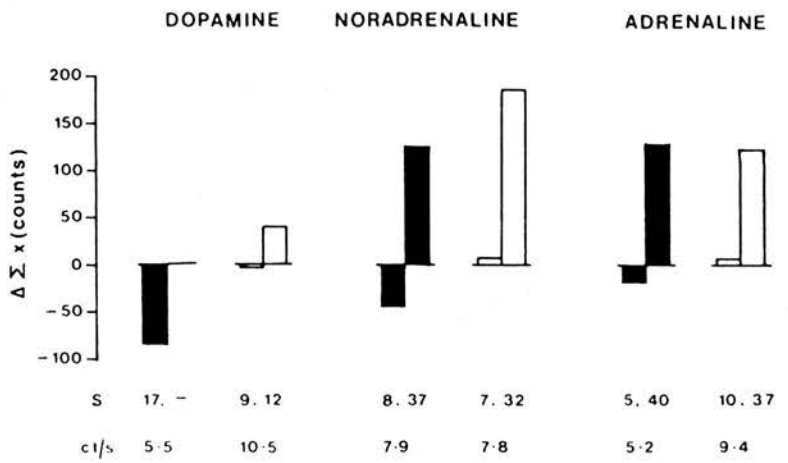


Fig. 7. Integrated response of chemoreceptors (3 units) recorded in one experiment to i.c. injections of catecholamines (5 μg) before (black blocks) and after (white blocks) (−)-sulpiride (0.2 mg/kg i.c.). Background discharge frequency is shown in ct/sec, and the blocks represent $\Delta\Sigma x$, the total increase or decrease in discharge from the control level occurring over a period whose duration is given. Note that the depression of spontaneous discharge associated with all three amines was virtually abolished by the dopamine antagonist, but the delayed excitation was unaffected, or potentiated. Responses to ACh, NaCN and CO_2–Locke solution were unaffected by sulpiride. Cat, anaesthetized with pentobarbitone, artificially ventilated and paralyzed with gallamine. Ganglioglomerular nerves cut. (McQueen, unpublished observations.)

establishing whether exogenously administered catecholamines have similar actions to those of the endogenous amines released within the tissues by physiological stimuli.

The observation that chemoexcitation can occur in response to dopamine under certain circumstances, taken together with results from experiments in which selective antagonists and other drugs were used, has led to suggestions that endogenous dopamine in the carotid body may be chemoexcitatory (Docherty, 1979), which is in accord with Lever and Lewis' (1959) suggestion that a carotid body catecholamine is involved in impulse initiation. Cardenas and Zapata (1980) proposed that dopamine has a dual action, inhibiting chemosensory discharge when released in response to mild stimulation of the carotid body, but increasing the discharge when released in greater amounts by stronger stimulation. Differences in the accessibility of drugs to different sites in the carotid body might explain some of the apparent differences observed between preparations and species (see Bisgard et al., 1979).

What kind of receptors are involved in the response(s) of the carotid body to dopamine? Sampson (1972) showed that the α-adrenoceptor antagonist phenoxybenzamine in a dose of 20 mg/Kg i.v. abolished dopamine-induced depression of chemosensory discharge of the cat carotid body in situ. This could be interpreted as meaning that chemodepression results from actions on an α-adrenoceptor. However, subsequent studies revealed that lower doses of phenoxybenzamine (3 mg/kg), which

were apparently adequate for blocking actions of noradrenaline on the systemic vasculature, have no effect on the dopamine-induced depression of discharge in cats, nor does the β-antagonist propranolol (Zapata and Llados, 1977; Llados and Zapata, 1978b). Higher doses (15 mg/kg i.v.) did reduce the depression, but also increased basal discharge frequency and caused a marked fall in systemic blood pressure (Llados and Zapata, 1978b). These authors interpreted their results as meaning that the reduction in the response to dopamine was a result of the hypotension, since it was reversed upon restoration of blood pressure. However, it could also be the case that high doses of phenoxybenzamine can antagonize dopamine receptors. Nishi (1977) also found similar effects with phenoxybenzamine and phentolamine, and concluded that α-receptors are not involved in the chemodepressant action of dopamine. This conclusion is supported by the finding that any inhibition evoked by noradrenaline is antagonized by dopamine antagonists, and not by phenoxybenzamine (see later) and particularly by results showing that drugs which are reasonably specific dopamine antagonists are capable of eliminating dopamine-induced depression of discharge.

Various dopamine receptor antagonists have been tested in several species and it has been established that haloperidol, trifluperidol, spiroperidol, chlorpromazine, α-flupenthixol and some ergot alkaloids (e.g. dihydroergotamine) are able to reduce or abolish the depressant action of dopamine (Zapata, 1975; Zapata and Llados, 1977; Nishi, 1977; Llados and Zapata, 1978a; Zapata and Larrain, 1978; Docherty and McQueen, 1978b, 1979; Bisgard et al., 1979) and there seems little doubt that chemoinhibition is mediated via a dopamine receptor. Some of the antagonists may have actions on other receptors (e.g. α, 5-HT), but in the doses usually used the consensus is that they are relatively specific. Similar chemoinhibitory effects are obtained with apomorphine (Zapata and Llados, 1977; Docherty and McQueen, 1978b), amantadine (Llados and Zapata, 1978a) and certain ergot alkaloids such as 2-Br α-erocryptine (Zapata and Larrain, 1978) and these effects are associated with activation of dopamine receptors.

One can enquire whether enough evidence exists to classify the dopamine receptor involved in chemoinhibition as D-1 (linked to adenylate cyclase) or D-2 (which does not stimulate adenylate cyclase) as described by Kebabian and Calne (1979) for central dopamine receptors. The answer seems to be yes there is, because the pharmacological profile of the receptor is compatible with it being a D-2 receptor. Many of the dopamine antagonists are not capable of distinguishing between D-1 and D-2 receptors, but (−)-sulpiride is regarded as being selective for the D-2 receptor in pituitary cells (Stoof and Kebabian, 1981) and it virtually abolishes the depression of chemosensory discharge associated with dopamine in the cat carotid body (Fig. 7). Binding studies in the carotid body should reveal whether this deduction concerning the D-2 dopamine receptor is correct.

Abolition of the dopamine depressant effect by selective dopamine antagonists is often associated with an increase in basal discharge, which accords with Osborne and Butler's (1975) hypothesis. However, much depends on the dose of antagonist used, and Docherty and McQueen (1978b) did not find any significant change in frequency using a dose of α-flupenthixol in cats which greatly reduced the chemo-

depressant action of dopamine, nor did Zapata (1975) using spiroperidol in vitro. Responses to sodium cyanide, hypoxia and hypercapnia are potentiated after administering some dopamine receptor antagonists although excitation caused by ACh is unaffected (Nishi, 1977; Docherty and McQueen, 1978b, 1979; Lahiri et al., 1980) which is compatible with dopamine exerting an inhibitory effect on the receptor complex. However, results have to be interpreted cautiously because these electrophysiological studies were performed in the absence of the putative efferent pathway - the carotid sinus nerve from which activity was recorded had been cut centrally.

It has been shown in anaesthetized cats that a slow intravenous infusion of dopamine causes hypoventilation and a reduced respiratory response to hypoxia which is greatly reduced by sectioning the sinus nerves (Nishino and Lahiri, 1981), and this was correlated with a depression of chemosensory discharge recorded from the sinus nerve (Zapata and Zuazo, 1980). In anaesthetized rabbits intracarotid injection of dopamine also depressed ventilation, an effect which was abolished by haloperidol, although the hypoventilation in response to dopamine was sometimes preceded by a transient increase in ventilation following higher doses (Matsumoto et al., 1980). Experiments in man (Bainbridge and Heistad, 1980) have shown that i.v. dopamine reduces respiratory minute volume (RMV), an effect which is abolished by haloperidol without there being any effect on RMV or p_aO_2 measured during either normoxia or hypoxia. These observations can be interpreted as demonstrating the inhibitory actions of dopamine on the carotid chemoreceptors in humans, but central actions of haloperidol may complicate matters. Boll et al. (1976) found that the increased RMV in response to acute hypoxia in man was significantly potentiated during treatment with chlorpromazine (75 mg daily) and they interpreted this as being the result of an increased chemoreceptor input to the CNS. Again, possible central actions via dopamine, 5-HT, α- or acetylcholine receptors complicate the interpretation, but all the observations are consistent with dopamine exerting an inhibitory action on peripheral chemoreceptors.

When the depressant action of dopamine has been blocked, an excitatory effect is sometimes unmasked which is resistant to dopamine antagonists and to various other blockers, including α-blockers (Fig. 7; Zapata, 1975; Nishi, 1977) and β-blockers (Jacobs and Comroe, 1968). The excitation caused by dopamine in dogs can be antagonized by D-tubocurarine (Bisgard et al., 1979) but not by the nicotine antagonist gallamine. The receptor mediating this might, as pointed out by Docherty (1980), be similar to that asssociated with dopamine-induced depolarization of neurones in the molluscan nervous system which is blocked by D-tubocurarine (Ascher, 1972). A specific ligand for this receptor is LSD (Drummond et al., 1978), so studies with LSD on the carotid body may be fruitful. Nishi (1975) noted that this substance stimulates cat carotid body chemoreceptors in vivo, but offered no explanation for the effect. It must be stressed that some authors consider the chemoexcitatory action of dopamine is non-specific (Zapata, 1975; Mills et al., 1978), and more studies are needed to show whether or not a dopamine receptor (apparently not D-2 and probably not D-1) is directly or indirectly involved.

Various drugs capable of affecting endogenous dopamine in other systems have been tested for their ability to modify carotid chemoreceptor activity. Amphetamine

has been reported to cause chemoinhibition in cats, an effect which can be blocked by α-flupenthixol (Docherty and McQueen, 1978b). This might mean either that amphetamine releases dopamine in the carotid body or alternatively that it acts as an agonist at dopamine receptors. In contrast, however, Llados and Zapata (1978a) found tyramine and amphetamine (even 2 mg i.c.) to be without effect in the same species, and they concluded that dopamine is not released by these agents, suggesting there is no uptake mechanism for dopamine in the carotid body. Some studies have been performed on the carotid body using 6-OH-dopamine (e.g. see Murphy and O'Regan, 1977; Zuazo and Zapata, 1978), but as is the case with reserpine, results are difficult to interpret and there is a need for better correlation between the pharmacology and biochemistry and effects on adrenergic nerves have to be separated from those on type I cells. There is some evidence favouring an uptake mechanism in chemoreceptor cells (see Kobayashi, 1975; Fidone et al., 1980), but whether or not it is specific remains to be established, and the possibility that species variation exists has to be considered.

Studies with monoamine oxidase inhibitors, which potentiate the inhibitory action of dopamine (Docherty, 1980), or the tyrosine hydroxylase inhibitor α-methyl-*p*-tyramine (Docherty and McQueen, 1978b) are difficult to interpret without biochemical data showing the effect of the drugs on catecholamine levels and turnover in the carotid body.

Binding studies using [^{3}H]spiroperidol in rabbit carotid bodies show that chronic sectioning of the carotid sinus nerve reduces specific binding by 64% compared to normal controls (Dinger et al., 1981b). This is interpreted as showing that dopamine receptors are present on afferent sinus nerve endings, although it could also mean they are associated with efferent nerve endings. The remaining binding sites are likely to be on chemoreceptor cells, sympathetic nerve endings, or blood vessels. However, the ratio of non-specific to specific binding is very high, and further studies are needed to substantiate these interesting initial findings. Whether the binding site(s) correspond to the dopamine receptor(s) will also need to be shown. The technique of binding/displacement of radioligands has much to offer, and if its resolution can be enhanced, may prove a very useful method for studying carotid body receptors.

2.2.3. Noradrenaline and adrenaline

The predominant effect observed following injection of these substances in many species is chemoexcitation preceded by a brief period of inhibition which is not a "flush" effect of the vehicle (see Fig. 7; Sampson, 1972; Llados and Zapata, 1978b). In dogs noradrenaline usually causes inhibition (Bisgard et al., 1979). The delayed increase in discharge parallels the systemic vasopressor action and is abolished by phenoxybenzamine (Sampson, 1972; Llados and Zapata, 1978b) in low doses which prevent the vascular effects. Responses to various chemoreceptor stimulants are unaffected by phenoxybenzamine (Sampson, 1972). Therefore, the conclusion reached by Joels and Neil (1963) that the excitation seen following catecholamine administration, or stimulating the sympathetic nerves to the carotid body, is related to vasoconstriction seems quite reasonable. However, Bisgard et al. (1979) found the excitation caused by noradrenaline in dogs could be blocked by D-tubocurarine, an

effect which is not due to block of nicotinic receptors. After α-blockade the depressant actions of adrenaline and noradrenaline are enhanced, reflecting the fact that they are normally partially masked by the excitatory component of the response. The chemodepression is blocked by dopamine antagonists (see Fig. 7; Zapata and Llados, 1977; Llados and Zapata, 1978b) so one could conclude that it results from direct or indirect actions of the amines that can be blocked by dopamine (D-2) antagonists in cats. The fact that there are two components to the noradrenaline and adrenaline responses explains why administration of these catecholamines has been associated with variable effects on chemosensory discharge and ventilation.

Given that the actions on α- and dopamine receptors can apparently account for all the actions of injected catecholamines in cats, it is perhaps surprising to find reports that β-adrenoceptor agonists and antagonists can affect chemosensory discharge under certain circumstances. Biscoe (1965) studied the cat carotid body in vitro and found that adrenaline, noradrenaline and isoprenaline increased discharge, an effect which in this preparation cannot be secondary to vasoconstriction, and the effect of these drugs was blocked by the β-blocker pronethanol. Responses to hypoxia and ACh were also reduced by the β-antagonist and spontaneous discharge was depressed. Eyzaguirre and Koyano (1965) and Eyzaguirre and Zapata (1968a) failed to detect any chemoexcitation with the catecholamines in the same preparation, and they attributed the reduction in sensory discharge with dichloroisoprenaline, a drug reported by Biscoe to be less effective than pronethanol, to nerve fibre block. Local anaesthesia is a well-known property of some β-blockers in higher doses, but whether it can be invoked to explain away Biscoe's results is another matter. Greene (1966) used respiratory changes in anaesthetized cats to provide an index of chemosensory activity in response to acute hypoxia, intracarotid nicotine or sodium cyanide. Since neither propranolol (1 mg/kg i.v.) nor phenoxybenzamine (10 mg/kg i.v.) affected the responses, the author concluded that noradrenaline appears not to be a chemical transmitter at the carotid body. Heistad et al. (1972) consider their data in man demonstrate that β-receptors are involved in a hyperventilatory response to noradrenaline and isoprenaline, but not to hypoxia, whereas Patrick and Pearson (1978) concluded that propranolol has no effect on the chemical control of breathing in man. Lahiri et al. (1981) used anaesthetized cats and report that isoprenaline can stimulate chemoreceptors (see also Llados and Zapata, 1978b; Wasserman et al., 1979; Folgering et al., 1980) but that the magnitude of the response depends very much on the tension of CO_2 and O_2 in arterial blood (but cf. Wasserman et al., 1979). Propanolol (0.5 mg/kg i.v.) blocked the vascular effects of isoprenaline, and partially reduced the response to isoprenaline and to hypoxia. A slightly odd feature of the results is that increases in carotid chemoreceptor discharge began within 2.5 sec of an intravenous infusion and peaked at 5 sec, which is very rapid indeed for a direct action on the carotid chemoreceptors, given the time it takes for substances to pass from the vein to the carotid arteries via the pulmonary circulation. The authors conclude that the ventilatory effect of isoprenaline is mediated, in part, by peripheral chemoreceptors. However, increases in cardiac output can raise the P_aCO_2 and so increase chemosensory discharge, particularly in artificially ventilated animals. Isoprenaline can affect oxygen consumption, and β-agonists and antagonists can act within the CNS (Folgering, 1980), so interpretation of responses is complicated.

Folgering concluded from cat experiments that β_1-receptors in the brain stem stimulate the ventilatory control system: β_2-agonists (salbutamol) and antagonists (butoxamine) had no clear effect on respiration. Further studies using selective β_1- and β_2-agonists and -antagonists at different levels of P_aCO_2 and P_aCO_2, combined with binding studies, should establish whether β-receptors are present in the carotid body as is suggested by some, but not all, the pharmacological evidence presently available. If they are, it will be interesting to see whether they are associated with the vasculature or the chemosensory cells/fibres and to determine whether endogenous noradrenaline affects them directly or indirectly.

2.2.4. Conclusions

Depression of spontaneous and evoked chemosensory discharge by exogenous dopamine results from actions on a specific dopamine receptor, probably of the D-2 type, in the carotid body complex. The chemoexcitation associated with higher doses of dopamine, or seen under particular experimental conditions, appears not to be reduced significantly by α-, β- or dopamine antagonists that are commonly used. Whether it results from direct or indirect actions of dopamine, and whether these can be blocked by D-tubocurarine in species other than dog, possibly by antagonism at a dopamine receptor, remains to be established.

Noradrenaline and adrenaline commonly cause biphasic changes in chemosensory charge. Chemoinhibition caused by these amines seems to be mediated via dopamine receptors (D-2?). The increase in sensory discharge can be greatly reduced by α-blockers, and failure to elicit the excitatory response in vitro has led some authors to conclude that the response seen in vivo is the result of vasoconstriction within the carotid body. Others have reported that D-tubocurarine blocks the excitation without affecting responses to ACh or nicotine, which raises the possibility referred to above that a dopamine receptor is involved. However, D-tubocurarine may be affecting an ion channel since the drug is apparently capable of blocking excitation evoked by noradrenaline and 5-HT as well as dopamine. Biscoe's (1965) experiments on cat carotid bodies in vitro indicate that under the conditions of his experiments isoprenaline, adrenaline and noradrenaline can all increase discharge by affecting a β-receptor, which is supported by some, although not all, the data from other experiments with isoprenaline or β-blockers. It will be necessary to examine the chemoexcitatory effect of noradrenaline to see whether it involved actions at more than one site (i.e. a vascular effect involving α-receptors and some other action on the receptor complex involving a β-adrenoceptor or a D-tubocurarine-sensitive site).

The results obtained from pharmacological studies on the carotid body chemoreceptors depend on the doses of agonist and antagonist used, their specificity for a particular receptor, the physiological state of the preparation (i.e. whether normoxic or hypoxic; normocapnic or hypercapnic), the time for which the drugs act, the route by which they reach the receptor(s), and their effects may be modified by actions on the vasculature. It is not too surprising, given the complexity of the situation, that opposing results are obtained by workers studying drug effects in different preparations or species, particularly when using drugs such as α-blockers and dopamine

antagonists whose specificity in the doses employed is not always so great as is claimed. Conflicting data need not mean that real differences do in fact exist in different preparations or species.

Results obtained from pharmacological experiments do not provide an answer to the question of whether or not carotid body amines act as transmitters. They may function as modulators, increasing or decreasing the gain of the chemoreceptors, but they could also have other actions (e.g. hormonal, vascular) which influence chemosensory activity only indirectly, if at all.

2.3. Polypeptides

2.3.1. Historical review

Historical is not really a very appropriate word because much of the work on polypeptides is very recent and has yet to stand the test of time. Pearse (1969) classified the carotid body type I cell as a member of the APUD cell series (cells which share the characteristics of *a*mine and amine *p*recursor *u*ptake and *d*ecarboxylation, together with polypeptide secretion). He suggested that the type I cell secretes a low molecular mass polypeptide which was tentatively named "glomin". Histological evidence was subsequently obtained which indicated the presence of polypeptide- or protein-containing granules in mammalian carotid body cells (Capella and Solcia, 1971; Pearse et al., 1973), and Torrance (e.g. 1974) drew attention to the possible physiological significance of peptides in the carotid body.

McQueen (1978) showed that substance P (SP) can influence chemoreceptor activity, and at the 1979 International Arterial Chemoreceptor Meeting held in Valladolid evidence was presented (McQueen, 1981) demonstrating that various polypeptides, including SP and methionine enkephalin, can affect chemoreceptor activity in cats. Furthermore, at the same meeting Fitzgerald et al. (1981) reported, firstly, that vasoactive intestinal polypeptide (VIP) is present in the carotid bodies of cats and dogs, and secondly, that VIP could modify chemoreceptor discharge recorded from cats. The presence of VIP-like material in nerve fibres but not cells of the carotid body has been confirmed by immunohistochemistry (Lundberg et al., 1979a; Wharton et al., 1980).

The presence of SP-like immunoreactive material in some cells and nerve fibres of the cat carotid body has also been demonstrated by the immunofluorescent technique (Cuello and McQueen, 1980), and SP has also been reported to be present within nerve fibres, but not cells, in cat carotid bodies (Lundberg et al., 1979a; Wharton et al., 1980). The latter group also confirmed the presence of SP-like material in cat carotid body using radioimmunoassay. It is interesting to note that Hanbauer (1977) used a sensitive radioimmunoassay for SP but did not find any SP in rat carotid body, whereas Jacobowitz and Helke (1980) showed the presence of SP-like immunoreactivity in some nerve fibres of the rat carotid body. Radioimmunoassay and immunohistochemical techniques may provide different information because much depends on the specificity and type of antibody used, the concentration of antigen in the tissues, and the methods used for preparing the tissues. Differences in antibodies or methods employed may also explain why Cuello and McQueen (1980) detected

SP-like material in carotid body cells, whereas other workers did not, but it has also to be realized that the antibodies may cross-react with peptides which are as yet unidentified (e.g. an SP-precursor molecule), so it has to be stressed that what is being detected is *SP-like* material, not necessarily SP. This sometimes seems to get overlooked when discussing and interpreting results.

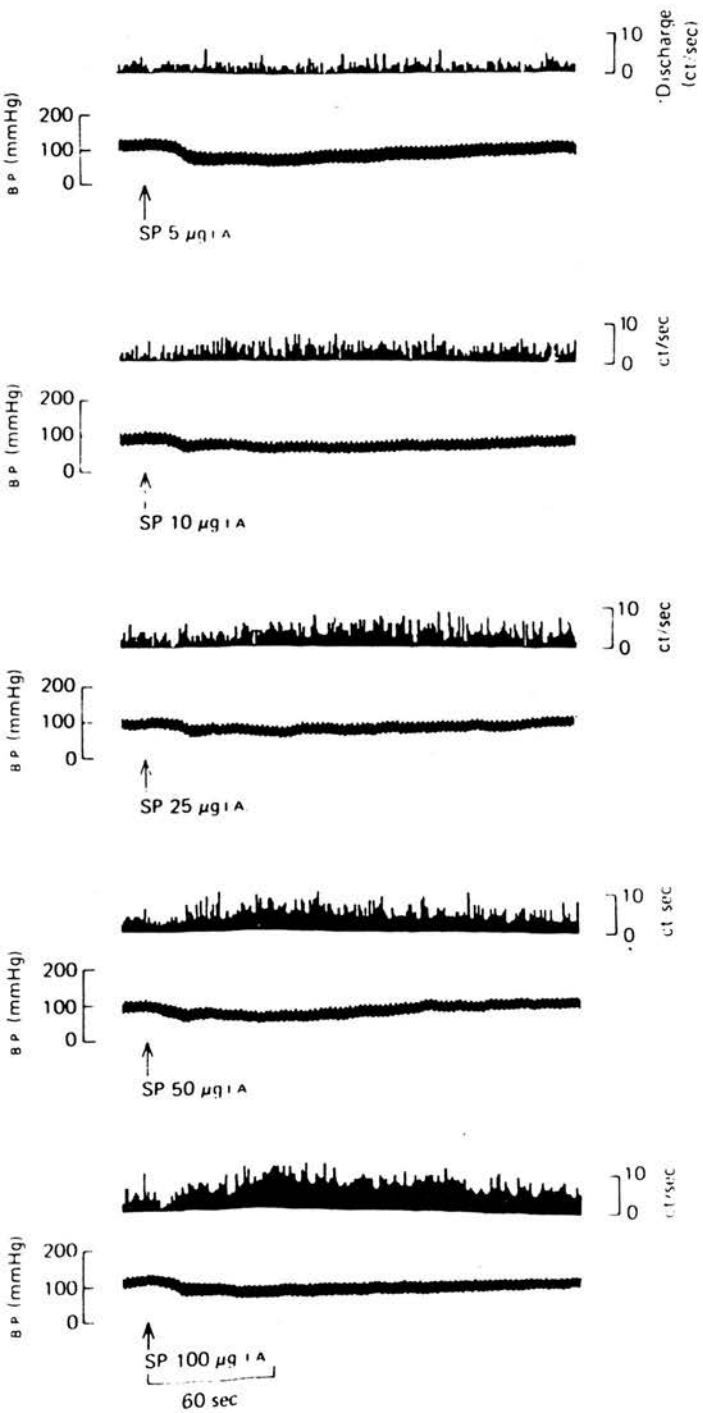
It has recently been suggested that SP is a central neurotransmitter of chemoreceptor afferent fibres (Haeusler and Osterwalder, 1980; Helke et al., 1980; Jacobowitz and Helke, 1980), and if this were proved to be the case, it might mean that SP in the carotid body is associated with the peripheral endings of primary afferent chemoreceptor fibres (i.e. applying the "Dale principle", but in reverse direction – Dale, 1935). The fact that SP-like material has been found in the petrosal ganglion (Lundberg et al., 1979b) is in accord with this suggestion.

Enkephalin (ENK)-like immunoreactive material has also been detected in cells but not nerves of the cat carotid body (Lundberg et al., 1979a; Wharton et al., 1980). Both methionine-ENK and leucine-ENK have been identified in carotid body extracts by radioimmunoassay, there being 3–4 times more met- than leu-ENK (Wharton et al., 1980). The particular type(s) of cell with which this, or any of the other polypeptides, is associated in the carotid has yet to be established, and the same applies to the nerves in which some of the peptides are present.

Polypeptides may act as neurotransmitters, but evidence relating to such a function is not overwhelming, and they could have a neuroendocrine function (Meites et al., 1979). Although the question of what physiological role they perform is still a matter for conjecture and debate (e.g. see Guillemin, 1978; Fujita and Kobayashi, 1979; Hökfelt et al., 1980), peptides have been included in this review partly because some are present in the carotid body and can modify chemoreceptor activity, and partly because of evidence from other parts of the nervous system which suggests they are neurotransmitters (see Hökfelt et al., 1980; Nicoll et al., 1980).

2.3.2. Substance P (SP)

SP affects spontaneous chemosensory discharge when injected close-arterial to the cat carotid body causing an initial slight depression followed by a dose-related increase in discharge (Fig. 8) (McQueen, 1980a). Compared with the effects evoked by injections of ACh or dopamine, the actions of SP are delayed, longer-lasting and less intense, and part of the response is secondary to a rise in P_aCO_2, possibly as a consequence of SP altering bronchoconstrictor tone or cardiac output. Difficulties are encountered when interpreting delayed effects in vivo because of the possibility that these are secondary to other actions of the drug, such as vascular effects, and although steps can be taken to avoid or compensate for such secondary effects, it does highlight the fact that there is much to be said for studying effects on the in vitro preparation as well as in vivo. Monti-Bloch and Eyzaguirre (1980) reported that SP reduces spontaneous chemoreceptor discharge recorded from the cat carotid body in vivo, and similar effects are apparently observed when carotid bodies from rabbits and mice are used (unpublished observations; see Eyzaguirre and Fidone, 1980). Disagreement between results obtained from carotid bodies in vivo and in vitro may, as previously discussed, reflect fundamental differences in the preparations or in the temporal


Discharge (ct/sec)
10
0
BP (mmHg)
200
100
0
SP 5 μg i.A.
SP 10 μg i.A.
SP 25 μg i.A.
SP 50 μg i.A.
SP 100 μg i.A.
60 sec

pattern of drug action, but could equally well be a reflection of the extent to which secondary factors in vivo modify the primary response of the chemoreceptors.

Changes in discharge evoked by substances such as ACh, sodium cyanide or dopamine are modified by SP (McQueen, 1980a). Responses to ACh and dopamine are reduced during SP infusion, whereas those to sodium cyanide are potentiated. It was concluded that some of these effects may be due to a blocking or modulating action of SP on ACh at nicotinic receptors, such as is known to occur on Renshaw cells (Ryall and Belcher, 1977). The delayed inhibition or depression of spontaneous chemosensory discharge that follows injection of 5-HT (see Fig. 11) was enhanced during SP infusion. It should be noted that Monti-Bloch and Eyzaguirre (1980) state in their abstract that low concentrations of SP had no effect on responses evoked by ACh in vitro, but high concentrations potentiated the stimulatory effect of ACh; the inhibitory effect of dopamine was converted to an excitation in the presence of low concentrations of SP. The absence of a full report makes it impossible to evaluate their findings.

The results obtained from studies with SP on the chemoreceptors are of a rather preliminary nature, and much remains to be done. For example, it has to be established whether the peptide affects cells/nerve endings (are A and C fibres similarly affected, or is there a differential action) in the carotid body, whether more than one receptor/action is involved and whether this is of any physiological significance. Is SP released by physiological stimuli, and if so, does it act on the same receptor(s) as exogenously administered SP? Some answers might come from studies with radiolabelled SP, or from labelling some of the amino acids which are incorporated into SP. It should be possible to detect SP in the superfusion fluid from carotid bodies by radioimmunoassay and to correlate its output with chemosensory activity – this would also provide evidence relating to the question of whether or not the time course of action of exogenous SP (seconds or minutes) is appropriate.

It might be argued that the relatively slight long-lasting effects evoked by exogenous SP is evidence against endogenous SP having a transmitter role. However, it is possible that exogenous SP does not reach an effective concentration at an intrinsic site (e.g because of enzymatic destruction) or affects other non-specific sites in such a way as to mask the "primary" effect. Further pharmacological studies might resolve some of the problems, but SP is a difficult drug to study because: (a) it can cause tachyphylaxis, (b) there is no specific antagonist to it at present, (c) no drugs are known which selectively affect its biosynthesis or destruction, and (d) it might affect the release and/or synthesis of substances such as dopamine and 5-HT in the carotid body, as it does in the CNS (e.g. Carlsson et al., 1977; Reubi et al., 1978). SP may be stored with 5-HT in the carotid body, as it is in some neurones in the CNS

Fig. 8. Response of a single cat chemoreceptor unit to various doses of SP injected i.c. in random order, with at least 20 min between successive doses. Each panel shows a block diagram of chemoreceptor discharge in ct sec and femoral arterial blood pressure. It can be seen in this particular experiment that although a similar fall in BP was evoked by all the doses of SP, the delayed increase in chemoreceptor discharge was dose-dependent. (Reproduced from McQueen (1980a), with permission.)

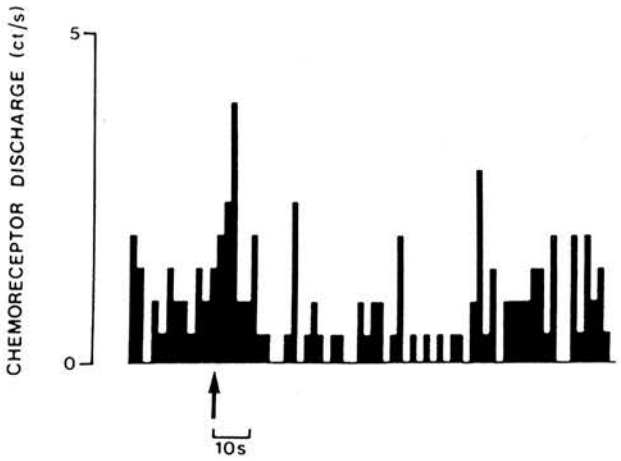


Fig. 9. Effect of an injection of capsaicin (1 mg i.c at arrow) on carotid chemoreceptor discharge recorded from a cat anaesthetized with pentobarbitone, artificially ventilated and paralyzed with gallamine. The ganglioglomerular nerves were intact. This was a lethal dose, and the cat died about 60 sec after the injection. (McQueen, unpublished observations.)

(Hökfelt et al., 1978). Capsaicin can be used to cause the release of SP within the carotid body (see Virus and Gebhart (1979) for a review on capsaicin, which is the active principle of hot peppers). Low doses of capsaicin (10 μg) had only slight effects on spontaneous chemoreceptor discharge when injected close-arterial to the carotid body (McQueen, unpublished observations), and although a high dose did cause some chemoexcitation (Fig. 9), it also killed the cat. Much may depend on the type of nerve fibre being studied – capsaicin is particularly effective on unmyelinated afferent fibres. In any event, it will be necessary to relate changes in discharge to SP output from the carotid body before any meaningful interpretation could be applied.

The evidence relating to the effect of SP on carotid chemoreceptors is very preliminary and does not really enable conclusions to be drawn concerning its role in the carotid body. Indeed, it remains a possibility that the substance present in the organ is not SP, but a very similar unidentified substance which cross-reacts with antiserum to SP. The lack of a pronounced chemoexcitation following injection of SP does not fit at all well with this peptide having an excitatory transmitter role in the carotid body. It might be argued that exogenous SP exerts a dual action, with chemo-inhibition masking the chemoexcitation. However, this is an argument that could be applied to any putative transmitter which has only slight effects on chemosensory discharge, and it seems somewhat artificial and suspect. In other systems reports that SP stimulates sensory nerve endings (e.g. Juan and Lembeck, 1974) have not been confirmed (Lembeck et al., 1977). If it should transpire that SP is not a sensory transmitter, and it must be emphasised that this is mere speculation, what other functions might it have in the carotid body? It may be a transmitter in an efferent pathway, a co-transmiiter (Hökfelt et al., 1980), a modulator acting to modify the sensitivity of nerve endings or cells, a trophic factor, a hormone, or an agent

concerned with regulating blood flow in the carotid body. Caution should be applied when interpreting the results of experiments in which the carotid sinus nerve is stimulated during studies on the efferent pathway in case SP is released from sensory fibres by antidromic nerve activity, as occurs from some fibres in the skin (Hökfelt et al., 1977), and acts to alter blood flow and/or chemosensory activity.

Further studies are needed to determine the precise localization of SP or SP-like material in particular elements of the receptor complex, to characterize the SP receptor(s), and to investigate the biophysical consequences of receptor activation. Some information concerning the role of SP in the carotid body may also come from studying animals which have been treated with capsaicin shortly after birth since this procedure mainly destroys unmyelinated primary afferent nerves (Jancsó et al., 1977), particularly those containing SP (Gamse et al., 1980) – although other peptides may also be affected (e.g. somatostatin – Keeler and Black, 1981).

2.3.3. Enkephalins and β-endorphin

Met- and leu-ENK are approximately equipotent in causing a rapid and pronounced dose-related depression of spontaneous discharge recorded from the cat carotid body in vivo (see Fig. 10) (McQueen and Ribeiro, 1980). There is no detailed evidence relating to the effects of the peptides on the carotid body preparation in vitro, only an abstract by Monti-Bloch and Eyzaguirre (1980) stating that met-ENK increases chemosensory activity. Whether there is in fact a real difference between the actions of ENK in vivo and in vitro must await the publication of a full report of experiments in vitro. As far as β-endorphin (β-END) is concerned, it too inhibits chemosensory discharge in vivo (see Fig. 10) (McQueen and Ribeiro, 1981b), but the effect tends to be bi- or tri-phasic, with excitation following the initial depression of discharge. Its actions are, overall, more like those of morphine than ENK (McQueen and Ribeiro, 1980).

Responses evoked by injecting ACh or CO_2–Locke solution were slightly potentiated during i.c. infusions of met-ENK, whereas the chemoexcitation caused by sodium cyanide was reduced (McQueen and Ribeiro, 1980). Tests performed during i.c. infusion of β-END revealed a reduction in responses to ACh, CO_2–Locke and to sodium cyanide, but a potentiation of the dopamine-induced depression of chemosensory discharge (McQueen and Ribeiro, 1981a). It is difficult to know how much reliance can be placed on these results because only single doses of the test substances were investigated, but they do show that opioid peptides are capable of modifying evoked responses as well as spontaneous chemoreceptor discharge.

Low doses of the opiate antagonist naloxone greatly reduce or abolish the chemoinhibition caused by β-END, and indeed its effect becomes predominantly excitatory (Fig. 10) (McQueen and Ribeiro, 1981a). The inhibition associated with ENK is also reduced by naloxone, but not to the same extent, and even additional high doses of naloxone do not entirely eliminate chemoinhibition (Fig. 10) (McQueen and Ribeiro, 1980, 1981b) although the overall effect, particularly in response to low doses of met-ENK, may become excitatory. Naloxone itself has only a very slight effect on spontaneous chemosensory discharge (McQueen and Ribeiro, 1980). These observations could be interpreted as meaning that β-END causes chemoinhibition by

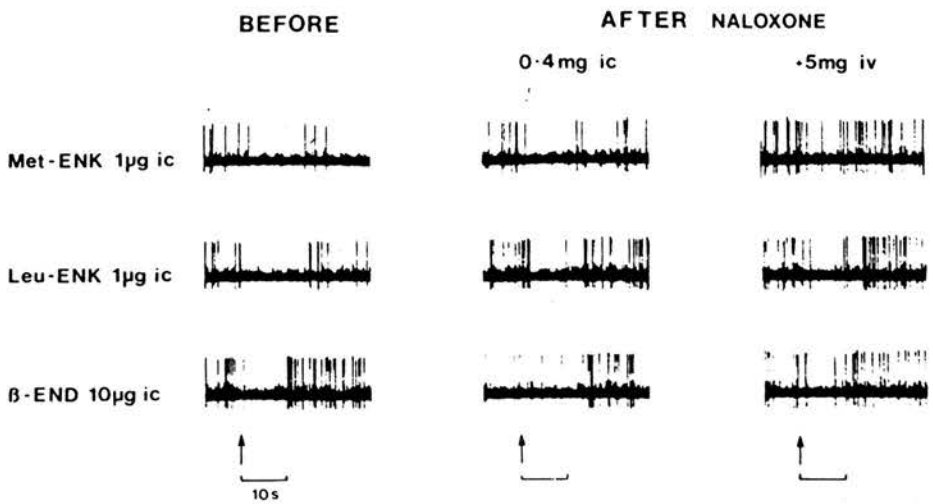


Fig. 10. Responses of a single chemoreceptor unit to intracarotid injections (arrows) of enkephalins and β-END before and after naloxone at 2 dose levels. Cat anaesthetized with pentobarbitone, artificially ventilated and paralyzed with gallamine. Ganglioglomerular nerves cut. (From McQueen, 1983, with permission.)

acting on a μ-receptor which is readily blocked by naloxone (see Lord et al., 1977, or Pert et al., 1980, for discussion on different types of opiate receptor) whereas ENK inhibits by affecting a δ-receptor which is more resistant to naloxone. The excitation seen with β-END, and some doses of ENK after naloxone, may be due to actions on an opiate receptor which is naloxone-insensitive (κ?), or to direct or indirect actions of the peptides on other receptors. Much depends on the specificity of naloxone as an opiate receptor antagonist, and it has been pointed out by Sawynok et al. (1979) that it may not be quite so specific as some have assumed, particularly when used in high doses.

The evidence is still rather preliminary, but it allows us to conclude that one or more opiate receptors are present in the carotid body, that the predominant effect of leu- and met-ENK is chemoinhibitory, but a slight excitatory action can be unmasked by naloxone in vivo. In other parts of the nervous system evidence has been obtained which suggests that ENK and β-END affect Ca^{2+} uptake in excitable tissue, and this action may lead to a depression of transmitter release (e.g. Guerrero-Munoz et al., 1979). It has been shown that chemoinhibition caused by ENK, although somewhat similar to that caused by dopamine, is not appreciably affected by the dopamine antagonist α-flupenthixol (McQueen, 1981) nor is the inhibition evoked by dopamine antagonized by naloxone (McQueen and Ribeiro, 1980). SP was unable to overcome or prevent the chemoinhibition caused by an intracarotid infusion of met-ENK (McQueen and Ribeiro, 1981b).

How these peptides act to modify chemoreceptor activity, and the receptors and biophysical/biochemical changes involved (e.g. is the effect linked with changes in

cAMP — see Snyder, 1980), all remains to be established. It will be necessary to perform further studies in order to determine whether opiate receptors in the carotid body are affected solely by endogenous opioids or whether circulating opioids released from the adrenal glands (e.g. Costa, 1980; Viveros et al., 1980) or by sympathetic nerves (Wilson et al., 1980; Konishi et al., 1981) also affect them. Whether ENK in the carotid body is stored with noradrenaline, as occurs in the adrenal medulla (Schultzberg et al., 1978), how endogenous opioids are released within the carotid body, and which elements within the receptor complex are involved all needs to be investigated, and recent work on the adrenal medulla may provide some useful insight into the role of these peptides in the carotid body (see Viveros et al., 1980). Could opioids act as modulators or as co-transmitters with other substances in the carotid body? Chemosensory responses to physiological stimuli are not appreciably affected by naloxone and naloxone has only slight effects on spontaneous chemoreceptor discharge under normoxic conditions according to the results obtained to date. This may mean that if opioids in the carotid body have a transmitter function, it is associated with an efferent pathway: it will be interesting to see whether naloxone can block efferent inhibition of chemoreceptor discharge.

2.3.4. Vasoactive intestinal polypeptide (VIP)

VIP was shown by Fitzgerald et al. (1981) to cause a slight increase in spontaneous chemosensory discharge when infused (5–25 μg in 0.5 ml over 10 sec) close-arterial to the cat carotid body in vivo. McQueen and Ribeiro (1981a) confirmed that an increase in discharge occurs which is sustained throughout a 5 min infusion of VIP (0.5 μg/min) into the carotid body. They also showed that injections of low doses of VIP depress spontaneous chemoreceptor discharge whereas higher doses increase discharge. Responses evoked by ACh, sodium cyanide, or CO_2-equilibrated Locke solution were reduced during infusions of VIP, as was the depression of discharge caused by met-ENK (10 μg i.c.).

The effects of VIP on spontaneous discharge are not as intense or dramatic as those observed following an injection of ACh or sodium cyanide, but it has to be remembered that polypeptides have a relatively high moecular mass compared with the other substances and, furthermore, may be extensively inactivated before reaching the "receptor" site. VIP affects the vasculature, causing a fall in systemic blood pressure, and it will be necessary to perform experiments in vitro to confirm the impression (McQueen and Ribeiro, 1981a) that vascular actions of VIP are not responsible for the greater part of the chemoreceptor response.

VIP or a closely related material appears to be present in some cholinergic neurones (Lundberg et al., 1979b) but whether VIP in the carotid body co-exists with ACh in nerve fibres (A and/or C?) has yet to be investigated as has the question of whether VIP stimulates formation of cAMP in the carotid body, as it does in brain tissue, by activating adenylate cyclase. VIP is thought to function as a neurotransmitter or neuromodulator and it has been hypothesized that VIP may be released from the same nerve endings as ACh in sweat glands (see Hökfelt et al., 1980). Further studies on the actions of VIP in the carotid body must be performed before any conclusions can be reached regarding its function in the organ.

2.3.5. Various other polypeptides

The number of polypeptides known to be associated with nerves and other cells in the body has increased dramatically over the last decade. Some of them are considered to be transmitters in other parts of the nervous system (see Hökfelt et al., 1980) and consequently it seemed worth examining their actions on the carotid chemoreceptors: most of them do modify spontaneous chemosensory discharge when injected in fairly high doses close-arterial to the carotid body, but whether this has any physiological significance is another matter. The effects of neurotensin, somatostatin, bradykinin, angiotensin II and vasopressin (ADH) were described by McQueen (1981) and results obtained from studies with CCK-8 on cat carotid chemoreceptors have also been reported (McQueen and Ribeiro, 1981a). The majority of peptides so far examined, including carnosine, glucagon and bombesin (McQueen, unpublished), do not produce any marked change in spontaneous chemoreceptor discharge, and it remains to be established whether the effects observed are due to primary actions of the peptide on the chemoreceptors, or are secondary to other actions (e.g. vascular effects).

2.3.6. Conclusions

More than 20 neuropeptides have been identified and it is likely that many more will be discovered. It therefore becomes a problem knowing which of these to study on the carotid chemoreceptor. The best policy may be to continue screening all neuropeptides (e.g. motilin and avian pancreatic polypeptide (APP) have not yet been tested on the chemoreceptors) but to study in depth only those which are shown to be present in the carotid body or for which receptors exist in this organ (i.e. circulating peptides may influence the chemoreceptors). It could also be worth investigating whether the carotid body contains a unique peptide, in addition to those already identified but known to be present in other parts of the body.

The results obtained so far do not enable any conclusions to be drawn concerning the role of peptides in the carotid body. They might function as transmitters, co-transmitters, neuromodulators, trophic factors, hormones, or agents controlling the vasculature, and may act either directly or indirectly by influencing the biosynthesis, storage or release of substances, including other peptides, in the carotid body, and could have more than one action. This must all remain mere speculation until studies are performed to investigate the type(s) of nerve (sensory A or C/efferent – sinus or sympathetic) the peptides are associated with, the particular type of cell in which some peptides are located, the characteristics of the peptide receptor, and the biophysical and biochemical changes arising from receptor activation. It will also be necessary to correlate peptide release and synthesis with the response to physiological stimuli. Some of these studies should be fairly straightforward (e.g. selective denervation of the carotid body to reveal which nerves contain peptides, use of radiolabelled peptides or precursors) but others will be very difficult to perform with the techniques currently available. Advances in understanding the physiological role of peptides in the carotid body can be expected to come from the use of selective peptide antagonists or from drugs which selectively inhibit enzymes involved in destroying peptides, but for most polypeptides such drugs do not exist at present.

Problems associated with the interpretation of results obtained with colchicine, which blocks axon transport of peptides, or from the use of specific antibodies to "block" the activity of endogenous peptides, are likely to limit the usefulness of these approaches, but experiments on cells of the carotid body and nerves (from petrosal ganglion, superior cervical ganglion) grown in tissue culture may prove to be rewarding.

2.4. Other putative transmitters

Various substances referred to in section 1 will be reviewed very briefly in this section. *5-Hydroxytryptamine* (5-HT; serotin) is present in the carotid body and when injected close-arterial to the carotid body in situ it affects chemosensory discharge (Fig. 11) (Black et al., 1972; Nishi, 1975; Docherty and McQueen, 1978b, 1979; Bisgard et al., 1979). The overall effect varies according to species studied, but a short-lasting increase in chemoreceptor discharge commonly occurs immediately on injecting 5-HT, and this is followed by a longer-lasting inhibition of spontaneous activity. By using very low doses of 5-HT it is possible to elicit only the inhibitory component, at least in dogs (Bisgard et al., 1979).

The response to 5-HT in cats is unaffected by atropine, hexamethonium, LSD, methysergide or gramine (Nishi, 1975) but the excitatory component can be abolished by low doses of the dopamine antagonist, α-flupenthixol (Docherty and McQueen, 1978b) – higher doses of this antagonist also blocked the depressant component. In dogs the excitatory effect of 5-HT is reduced by D-tubocurarine and the inhibitory response blocked by dihydroergotamine (Bisgard et al., 1979). The results obtained with D-tubocurarine in dogs need to be verified and extended to other species, but they appear to show that noradrenaline, dopamine and 5-HT cause chemoexcitation via a common mechanism that can be blocked by D-tubocurarine,

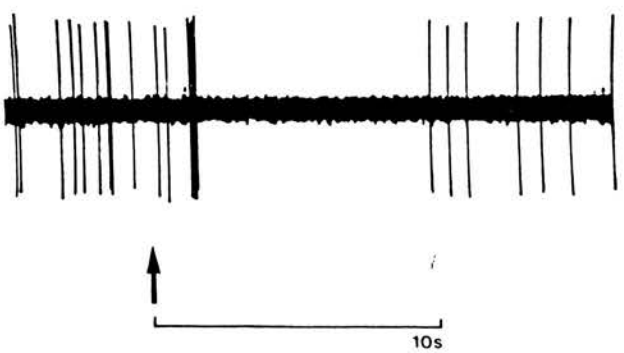


Fig. 11. Single chemoreceptor unit showing the biphasic response (excitation followed by inhibition) to an intracarotid injection of 5-HT (10 μg) (arrow). Cat, anaesthetized with pentobarbitone, artificially ventilated, paralyzed with gallamine. Ganglioglomerular nerves cut. (Based on Docherty and McQueen, 1978b.)

but does not involve a nicotine-sensitive ACh receptor. Responses to physiological stimuli are unaltered by doses of D-tubocurarine which abolish the excitatory action of the amines. Is 5-HT acting directly, or is it releasing one of the other carotid body amines, or some other substance? The lack of specific 5-HT antagonists and the probable existence of more than one type of 5-HT receptor (Gaddum and Picarelli, 1957) complicate the pharmacological studies, and the fact that 5-HT is reported to have no significant action on carotid body chemosensory activity in vitro (Eyzaguirre and Koyano, 1965) could be taken to mean that some or all the responses obtained with 5-HT in vivo are secondary to vascular effects. However, the failure to demonstrate effects in vitro could equally well be related to deficiencies in the superfused preparation or the techniques used for applying the amines.

Although the present evidence is insufficient to support a transmitter role for 5-HT in the chemosensory mechanism, further pharmacological and biochemical studies are needed to establish whether or not 5-HT is released in response to physiological stimuli and to characterize the location and type(s) of receptor involved. An interesting prospect also worth investigating is that 5-HT acts as a co-transmitter with one of the carotid body peptides. Rapid advances can be anticipated once specific 5-HT antagonists and uptake blockers become available, possibly in the very near future.

There is virtually no evidence to support the candidature of *prostaglandins*, *histamine* or *amino acids* as putative transmitters in the carotid body. Prostaglandins A_2, E_2 and $F_{2\alpha}$ do have some effects on spontaneous chemosensory discharge recorded from the cat carotid body in situ, but no action could be detected in vitro (McQueen and Belmonte, 1974). The responses observed in vivo may, therefore, have been secondary to the well-known vascular effects of prostaglandins. Similarly, the balance of evidence suggests that neither histamine, antihistamines (Liljestrand, 1954) or various amino acids (Fig. 12) have any appreciable effect on spontaneous chemo sensory discharge when injected close-arterial to the carotid body. Most of these studies have been performed against normal or "resting" chemosensory activity, and there may be a case for a more detailed investigation under conditions of hypoxia or hypercapnia. Furthermore, the lack of effect on chemosensory activity may be due to an insufficient concentration of drug at the "receptor" site, and experiments should be performed either applying substances by ionophoresis (e.g. in slice preparations), or using selective antagonists (where these are available (e.g. to amino acids, Davies et al., 1980), in order to establish whether or not the receptors exist. This may seem rather unnecessary given that the lack of effect of these substances on chemoreceptor activity can more simply be interpreted as meaning that they are not involved in chemoreception. However, as long as the transduction mechanism remains unknown, it is worth continuing to explore all the possibilities, par-particularly since some amino acids are present in the bovine carotid body (Thörn, 1981).

Finally, *adenosine* and *ATP* can excite chemoreceptors when injected close-arterial to the cat carotid body (Fig. 13) (Jarisch et al., 1952; Dontas, 1955; Anichkov and Belen'kii, 1963) and ATP is present in type I cells of the cat carotid body, apparently stored together with catecholamines (Böck, 1980). The Russian authors developed a theory of chemoreception based on the balance of energy rich phosphate bonds in the

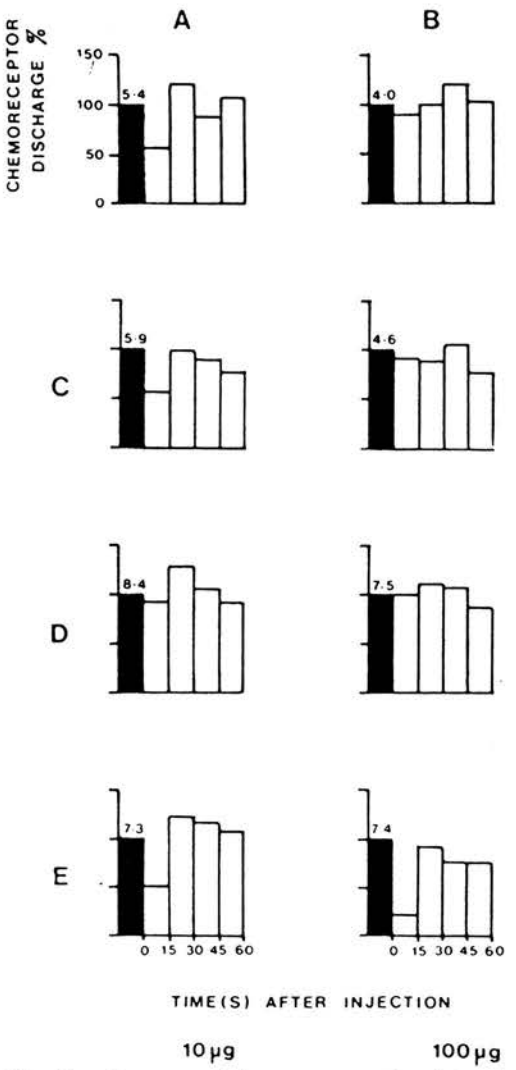


Fig. 12. Response of chemoreceptors (2 units) to intracarotid injection of: A, 0.3 ml unbubbled Locke solution; B, 0.3 ml Locke solution equilibrated with 5% CO_2:95% air illustrating that the transient inhibition caused by unbubbled Locke is due to the low PCO_2 of the solution. Responses to injection of 10 and 100 μg of sodium glutamate (C), GABA (D) and taurine (E) are also shown. Taurine and glutamate have been identified in the bovine carotid body, together with much lower concentrations of GABA (Thörn, 1981). Discharge was averaged over 15 sec periods and expressed as a percentage of the pre-injection discharge (black rectangle = 100%. Absolute value given in ct/sec). Cat, anaesthetized with pentobarbitone, artificially ventilated and paralyzed with gallamine. Ganglioglomerular nerves intact. (McQueen, unpublished observations.)

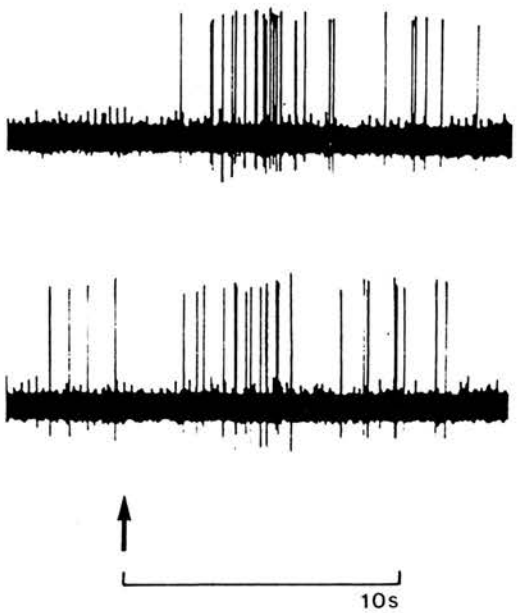


Fig. 13. Single chemoreceptor unit showing the responses to injections (arrows) of adenosine (1 μg i.c., upper panel) and ATP (1 μg i.c., lower panel). Cat, anaesthetized with pentobarbitone, artificially ventilated, paralyzed with gallamine. Ganglioglomerular nerves cut. (McQueen and Ribeiro, unpublished observations.)

carotid body (Anichkov and Belen'kii, 1963), a fall in ATP content of this organ leading to chemoexcitation by an unspecified mechanism (see Duncan, 1965; but cf. Joels and Neil, 1968). It is debatable whether exogenously administered ATP would appreciably affect intracellular levels of the nucleotide, and an alternative explanation is that the effects result from actions on extracellular adenosine receptors (see McQueen and Ribeiro, 1981b) following the breakdown of ATP to adenosine. Vascular effects of ATP and adenosine complicate matters. Again, it is impossible to judge whether ATP or adenosine is a carotid body transmitter or modulator since there is insufficient evidence. Further studies are needed, involving drugs that selectively affect the uptake of purines or block purinoceptors, and also biochemical measurements to show the relationship between ATP and chemoreceptor discharge in response to a varieity of physiological and pharmacological stimuli. The possibility that ATP functions as a co-transmitter, operating with one of the catecholamines and/or a peptide is intriguing and could be investigated.

3. Summary

Pharmacological studies have provided support for the suggestion that some of the substances reviewed in this chapter function as "modulators" of carotid chemosensory activity, but there is no really overwhelming evidence that any of them are

chemosensory transmitters. This could mean that chemical transmission is not involved in the transduction mechanism, but alternative interpretations are that either the experiments have failed to demonstrate a transmitter role for those putative transmitters which have been tested, or the transmitter is a substance which is, as yet, unidentified. The carotid body is a small structure and quite difficult to study. Variations between species, preparations and investigators, coupled with vascular and other non-specific drug effects, complicate matters. More emphasis needs to be given to correlating drug responses under different physiological conditions with biochemical (e.g. in levels of putative transmitters, adenylate cyclase, cAMP, cGMP) and biophysical (e.g. Na^+, K^+, Ca^{2+} conductance) changes, and to providing quantitative evidence.

The fact that substances regarded as transmitters in other parts of the nervous system have been identified within the carotid body leads to their being classified as putative transmitters in this organ. However, the possibility that they have some other role(s) (e.g. secretory, hormonal, trophic) should not be overlooked, for although the carotid body undoubtedly has a chemoreceptor function, it could also have others.

Pharmacological studies have provided some information regarding the different types of receptor present in the carotid body, and this may provide a clue regarding the normal ligands for the receptors and their function. However, it is necessary to proceed cautiously because exogenously administered putative transmitters may act at sites not normally affected by the endogenous substance, or be present in concentrations which are either too low or too high compared with physiological levels. In any event, receptors should not be classified on the basis of their responses to exogenous substances, but according to their natural ligands. This means establishing what the natural ligand is under physiological conditions, bearing in mind that it may come from within the carotid body, or reach the organ via the circulation (i.e hormone).

At present we do not know which of the elements in the receptor complex (cells or nerves) is primary, and perhaps the transduction mechanism involves the whole complex, such that it may prove impossible to investigate the role of individual elements in isolation (e.g. in tissue culture). Separate mechanisms might operate in the chemosensory mechanism (e.g. for A and C fibres, or even within a glomerulus) possibly using distinct transmitters or co-transmitters. The fact is that we do not know how transduction takes place in the chemoreceptors, but a lot of information, much of it from pharmacological experiments, has accumulated and provides a reasonable base for further studies on this fascinating subject.

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